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## Preliminary Findings of Contaminant Screening of Maine Bird Eggs, 2008 Field Season

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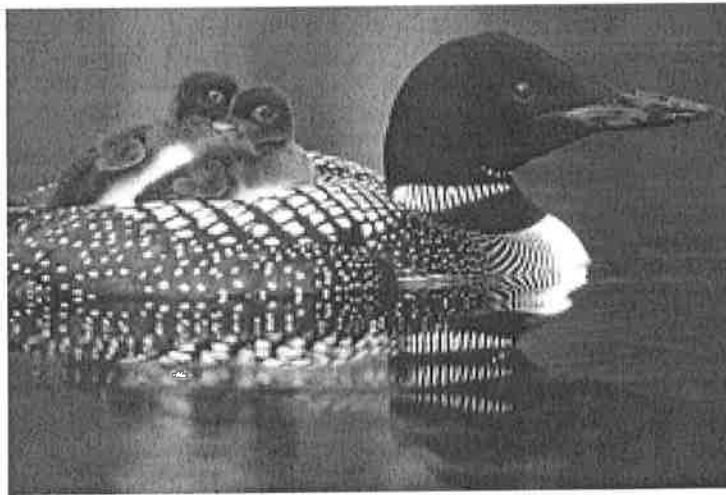
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## **Preliminary findings of contaminant screening of Maine bird eggs**

2008 Field Season



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# Preliminary findings of contaminant screening of Maine bird eggs: 2008 Field Season

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## 1. EXECUTIVE SUMMARY AND PRIMARY FINDINGS

Starting in May 2008, BioDiversity Research Institute (BRI) and collaborators expanded upon the 2007 broad-based contaminant study on Maine birds, measuring both historical and emerging chemicals. Out of the 23 species studied in the first year, we determined that three required additional study in 2008: common loon (*Gavia immer*), peregrine falcon (*Falco peregrines*), and piping plover (*Charadrius melodus*). We selected these species because loons act as bioindicators of lacustrine habitat throughout Maine, and peregrines and plovers are potentially at risk of bioaccumulating contaminants at levels above adverse effects thresholds. The compounds we analyzed in nine egg composites were mercury (Hg), polychlorinated biphenyls (PCBs, including coplanar congeners), polybrominated diphenyl ethers (PBDEs), perfluorinated compounds (PFCs), and organochlorine pesticides (OCs). Our preliminary findings are:

- Hg, PCBs, PBDEs, PFCs, and OCs continue to be detected in birds living in diverse habitats across Maine; PFCs were detected in all samples.
- Hg was detected in loons and peregrine falcons at levels above adverse effects thresholds.
- PFOS in common loons was detected at levels above adverse effects thresholds suggested for chickens. Androscoggin Lake had the highest level.
- The peregrine falcon sample from Mount Desert Island had the highest contaminant load, potentially from feeding on terns.
- Piping plovers continue to have contaminant levels higher than we expected for an invertivore.
- The loon samples did not show a specific spatial pattern, suggesting that within the lacustrine ecosystem, contaminant levels may be dictated by point sources, watershed characteristics, and/or food web dynamics.
- Like the 2007 results, PCB, PBDE, PFC, and OC levels are positively correlated, indicating that birds with high levels of one compound tend to have higher levels of the others. PBDEs and PCBs have one of the strongest relationships.
- DecaBDE is found in all three species, but not within each sample.

## 2. INTRODUCTION

### 2.1 Project overview

BioDiversity Research Institute (BRI) ran five common loon eggs, two piping plover clutches, and two peregrine falcon eggs for mercury (Hg), transformer coolants (PCBs, including coplanar congeners), flame retardants (PBDEs), stain repellants (PFCs), and organic pesticides (OCs). This analysis expanded upon our 2007 research that found over 100 toxic pollutants in 23 species of Maine birds (Goodale 2008). We focused on common loons because they are ideal bioindicators of lakes, have high levels of contaminants (Goodale 2008), have high levels of PFCs (Vogel pers. com.), have high levels of Hg (Evers et al. 2005), and have declining populations in Maine (Evers pers. com.). In fact, in recent years, reservoirs in Western Maine and New Hampshire have shown a dramatic decline in loon pairs: 30% on Mooselookmeguntic Lake (Taylor pers. com.), 58% on Lake Umbagog (Evers pers. com.), and 44% on Squam Lake in New Hampshire (Cooley pers. com.). The reason for these declines is not clear, and may be caused or be triggered by contaminants. We analyzed piping plover eggs because results from Goodale 2008 indicate that plovers had higher contaminant loads than expected and we analyzed two additional eggs from peregrine falcons. The falcon eggs were particularly valuable since there are only 23 nesting pairs in the state (C. Todd, Maine Department of Inland Fisheries and Wildlife, pers. com.).

### 2.2 Chemical Interaction

Researchers have studied the effects of many of the contaminants analyzed in this study on behavior, reproductive success, organ function, and acute toxicity. However, a number of studies have also attempted to determine if multiple compounds interact to create physiological effects greater than their sum. Researchers found that organochlorine pesticides can interact with each other to create either an additive or synergistic effect (Blus 2003). Epidemiological studies on human children (Grandjean et al. 2001, Stewart et al. 2003, Roegge et al. 2004), and laboratory studies on animals (Bemis and Seegal 1999, Costa et al. 2007) indicate that PCBs and methylmercury may act synergistically or additively. Additionally, researchers have found that PCB 52 can interact with PBDE 99 to enhance neurobehavioral defects in mice (Eriksson et al. 2006). These studies suggest that many of the compounds analyzed in this study can interact to create an effect greater than one contaminant alone.

### 2.3 Review of compounds measured

#### 2.3.1 *Hg*

Mercury is a naturally occurring heavy metal that has been mobilized into the environment by anthropogenic activities. Due to its unique properties, mercury is used in



many products such as thermostats and dental fillings. It is also used in mining, and is released to the environment through the combustion of fossil fuels.

Generally attributed to anthropogenic input (Lockhart et al. 1998), mercury (Hg) levels in the North Atlantic have doubled over the last 100 years (Asmund and Nielsen 2000) and are increasing by nearly 1.5% a year (Slemr and Langer 1992) with peak levels in Maine recorded after 1970 (Perry et al. 2005). This historical increase has been documented in North Atlantic seabirds (Thompson and Furness 1992, Monteiro and Furness 1997), Canadian Arctic seabirds (Braune 2007) with local Hg deposition causing high rates of increase in biota (Frederick et al. 2004, Evers et al. 2007). This increase of global Hg levels since the 1900s is of concern because mercury is a persistent toxic heavy metal that both bioaccumulates and biomagnifies<sup>1</sup> in wildlife, and has neurological and reproductive impacts (Wolfe et al. 2007).

Researchers have documented Hg in the Maine sediment (Perry et al. 2005), water (Dennis et al. 2005), crayfish (Pennuto et al. 2005), fish (Kamman et al. 2005), salamanders (Bank et al. 2005), birds (Evers et al. 2005), and mammals (Yates et al. 2005). In addition Hg hot spots have been documented in Maine (Evers et al. 2007).

### 2.3.2 PCBs

Polychlorinated biphenyls (PCBs) are synthetic chlorinated aromatic hydrocarbons that were first created in 1881; between 1930 and 1975 680 million kilograms were manufactured in the United States (Hoffman et al. 1996). Because of PCBs unique chemical properties they were used in a many industrial processes such as heat transfer agents, lubricants, dielectric agents, flame retardants, plasticizers, water proofing material, and most notably for cooling in electrical transformers (Hoffman et al. 1996). They are resistant to chemical breakdown, and have high thermal stability, low vapor pressure, flammability, and solubility (Niimi 1996). PCBs consist of two benzene (phenyl) rings connected by a carbon bond to which chlorine atoms are connected. The number of chlorine atoms provide the base for the 209 PCB congeners (Rice et al. 2003).

Originating from industrial leaks, sewage runoff, landfills, and incinerators, researchers have detected PCBs worldwide in the atmosphere, water, fish, birds, mammals, and humans (Hoffman et al 1996). Because of PCBs chemical structure, they are extremely persistent in the environment and resist being broken down by bacteria or chemicals. However, PCBs are easily absorbed into the fat of plankton and enter the food web (Hoffman et al 1996) and are eventually consumed by wildlife and humans.

In wildlife, PCBs both bioaccumulate and biomagnify. Piscivorous (fish eating) birds are most exposed to PCBs, and eagles and other top trophic level predators are particularly vulnerable to accumulating elevated levels. PCBs are extremely toxic to biota, causing wasting, immune effects, reduced reproduction, and liver damage (Hoffman et al 1996). In birds PCBs reduce egg hatchability, increase liver size, and affect thyroid and spleen

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<sup>1</sup> Builds up exponentially when one organism eats another.

function (Hoffman et al 1996). Researchers have observed similar effects in mammals with PCBs reducing reproductive success, and at high levels can lead to death (Kamrin and Ringer 1996). Because of these known effects, PCBs were banned in the United States in 1979 (Rice et al. 2003). Today in Maine PCBs are still widely detected in wildlife. They have been detected in mussels (Chase et al. 2001), seabirds, shorebirds (Mierzykowski and Carr 2004), eagle (Matz 1998), porpoise (Westgate et al. 1997), dolphin, and pilot whale (Weisbrod et al. 2001).

### 2.3.3 PBDEs

Polybrominated diphenyl ethers (PBDEs) are brominated flame retardants that are used in both commercial and residential textiles and electronics. They work by slowing combustion by releasing hydrogen bromide gas, which interferes with the chemical reaction that spreads fire (Janssen 2005). PBDEs consist of two benzene rings linked by an oxygen atom and can have up to ten attached bromine atoms (Hellstrom 2000). This stable structure causes the molecules to be lipophilic (fat loving) and consequently subject to bioaccumulation (Karlsson et al. 2006). The three primary types of PBDEs are penta-BDE, octa-BDE, and deca-BDE. Penta has been primarily used in polyurethane foam (up to 30% in weight) that is used in couches, carpets, and mattresses; octa is used in computer monitor plastics; and deca, which makes up 83% of global PBDE production, is used in electronic equipment (Johnson-Restrepo et al. 2005). Deca-BDE is an off-white crystalline powder that is usually 10-15% of the weight of the host material and is an additive flame retardant that does not chemically bond to its host material. Consequently, deca-BDE migrates into the environment (DEP 2007). PBDEs enter the environment through atmosphere deposition, wastewater treatment facilities, and runoff (Anderson and MacRae 2006).

PBDEs are found globally in humans, wildlife, and the environment. They have been found in whales, Tasmanian devils, fish, and falcons in Australia (Symons et al. 2004); terns in San Francisco Bay (She et al. 2004); guillemots in the Baltic Sea (Sellstrom et al. 2003); peregrine falcons in Sweden (Sellstrom et al. 2001); marine fish in Florida (Johnson-Restrepo et al. 2005); seabirds in Norway (Murvoll 2006); birds of prey in Belgium (Voorspoels et al. 2004); birds of prey in China (Chen et al. 2007); fish in Maine's Penobscot River (Anderson and MacRae 2006); and Arctic fox in Greenland and Russia (Lifgren 2005).

Laboratory studies have documented health effects of PBDEs, generally at levels higher than currently observed in the environment. Rats fed penta-BDE had reduced growth, diarrhea, reduced activity, tremors, red stained eye edges, and chewed continuously. Those animals that received repeated doses had changes in hepatic and thyroid size and histology as well as immunological effects. Rats fed octa-BDE had enlarged livers, and fetuses with bent ribs, limp bones, and rear limb malformations. Although health effects were observed at higher doses, animals dosed with deca-BDE had enlarged livers, and hyaline degeneration in kidneys. Those fed deca-BDE for 103 weeks at high doses developed tumors as well as an increase in thyroid, hepatic and pancreatic adenomas

(Darnerud 2003). A dosing study on kestrels found changes in thyroid levels and concludes: "Concentrations of PBDE congeners in wild birds may alter thyroid hormone and vitamin A concentrations, glutathione metabolism and oxidative stress (Ferne et al. 2005)." Because of these effects, penta and octa were voluntarily phased out in 2004 (EPA website), and deca was partially banned in Maine and Washington State in 2007.

#### 2.3.4 PFCs

Perfluorinated chemicals (PFCs) have been produced for over 50 years for their repellent properties and are used as stain repellents, cleaning agents, floor polish, fire-fighting foam, and in photography (Tao et al. 2006). Most commonly used PFCs are derived from perfluorooctanesulfonyl fluoride (POSF), which have extremely strong carbon-fluorine bonds. These strong bonds make the PFCs highly resistant to environmental and metabolic degradation (Butenhoff et al. 2006) and are consequently environmentally persistent (Kannan et al. 2002). Of the PFC congeners, perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) are of greatest concern because of their global abundance and bioaccumulation (Giesy and Kannan 2001, Kannan et al. 2002, and Tao et al. 2006).

Annual estimated production of POSF in 2000 was greater than 5000 tons (Tao et al. 2006), but by 2002 the 3M Company—the primary manufacture of POSF—discontinued production (Butenhoff et al. 2006). However, some PFOS is still produced outside of the United States for applications where there are no alternatives (Butenhoff et al. 2006) and other PFC are still produced and used in the United States (Kannan pers. com.). PFCs are transported in the environment through ocean currents and the atmospheric circulation (Toa et al. 2006) and may enter the environment through similar pathways as PBDEs.

Although there has been no analysis of PFCs in Maine, they have been documented in wildlife in the Southern Ocean and Antarctica (Toa et al. 2006), Arctic, North America, Pacific Ocean, Japan, Europe (Giesy and Kannan 2002), seaotters in California (Kannan et al. 2006), birds in Japan and Korea (Kannan et al. 2002), and in fish and pelicans in Columbia (Olivero-Verbel et al. 2006).

PFOS are documented to have health effects in wildlife. Hen eggs injected with PFOS had significantly lower hatching success (Molina et al. 2006). Quail exposed to PFOS through diet had increased liver weight and, at high levels, died (Newsted et al. 2007). In California, diseased sea otters were positively associated with elevated PFOS levels (Kannan et al. 2006).

#### 2.3.5 OCs

Organochlorine pesticides (OCs) are used primarily for insect control, are extremely persistent in the environment, and bioaccumulate in wildlife (Blus 2003). The five major groups are dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexane (HCH), cyclodienes, toxaphene, and chlordecone.

### 2.3.6 HCH

Hexachlorocyclohexane (HCH) is an insecticide that is currently used in agriculture—the most widely used form is lindane. Unlike other OCs pesticides, lindane has a short half-life and rapidly degrades after use. Consequently, lindane is rarely found in wildlife. However, in some laboratory studies lindane has reduced hatching success, increased embryo mortality, and caused egg shell thinning in chickens. In other studies researchers documented little effects (Blus 2003).

### 2.3.7 HCB

Hexachlorobenzene (HCB) is a fungicide used most commonly on seed grains, is an industrial waste product, and is used in the manufacture of tire rubber (Wiemeyer 1996). HCB is persistent in the environment and experimental studies have documented death and significant effects in birds. Quail fed high doses of HCB had weight loss, ruffling of feathers, and tremors. Birds fed a lower dose had reduced hatchability of eggs and sterile eggs (Wiemeyer 1996).

### 2.3.8 Chlordane

Chlordane is composed of number of OCs and has been used since the 1940s (Blus 2003). In 1978 most chlordane was restricted in the United States; all chlordanes are now banned (Wiemeyer 1996). The most toxic metabolite is oxychlordane (Wiemeyer 1996). In the past chlordane was used extensively on lawns, golf courses, and crops, and is persistent in the environment. The most measured effect in experimental settings is death. As recently as 1997 over 400 birds died from eating beetles with high chlordane residues in an area that had been treated in the past (Blus 2003).

### 2.3.9 DDT

Dichlorodiphenyltrichloroethane (DDT) was first synthesized in 1874, used as an insecticide in 1939, used extensively in agriculture after World War II (Blus 1996), and banned in the United States in 1972 (Blus 2003). Despite the well-documented effects on wildlife, DDT is still used in a number of countries. After application DDT breaks down to DDE. DDE has been well documented to cause egg shell thinning, which causes eggs to break during incubation. Because of the persistent nature of DDE, it is still widely detected in birds although at levels generally below effects thresholds (Blus 2003).

## 2.4 Birds as bioindicators of the environmental contaminants

Birds are commonly used as indicators of Hg and other contaminants in the environment (Scheuhammer 1987, Furness and Camphuysen 1997, Wolfe et al. 1998, Cifuentes et al. 2003, Braune 2007, Evers et al. 2005, and Sheuhammer et al. 2007, and Wolfe et al. 2007). The species we selected for this contaminant screening represent distinct foraging guilds and ecosystems across Maine. Additionally, some of the species we selected are high trophic level predators that may accumulate contaminants at higher levels. In total the 23 species of birds in our study indicate the contaminants other biota, and people—through consuming fish and game—may be exposed to.

## 2.5 Eggs as indicators of local contaminants

Eggs are used extensively for contaminant studies (Wiemeyer 1996, Kannan et al. 2001, Braune et al. 2002, Evers et al. 2003, and Braune 2007) because female birds depurate lipophilic contaminants into their eggs. For most species, all of the egg nutrients are allocated from exogenous (i.e. recent dietary uptake) rather than endogenous (reserves acquired during migration and on winter grounds) sources (Bond et al. 2007, Hobson 2006, Hobson et al. 2000, and Hobson et al. 1997). Consequently, egg contaminant residues represent the contaminants present in the bird's breeding territory diet (Hobson et al. 1997). These findings are supported by Evers et al. (2003), which found a strong relationship between common loon egg Hg levels and female Hg blood levels (blood represents recent dietary uptake). The exception is species that arrive on the breeding ground and immediately lay eggs (Hobson 2006). The species in our study are all present at their breeding site for at least two weeks prior to laying eggs (Table 1). Therefore, the results presented in this report represent contaminant levels of the birds within their foraging range during the breeding season in Maine.

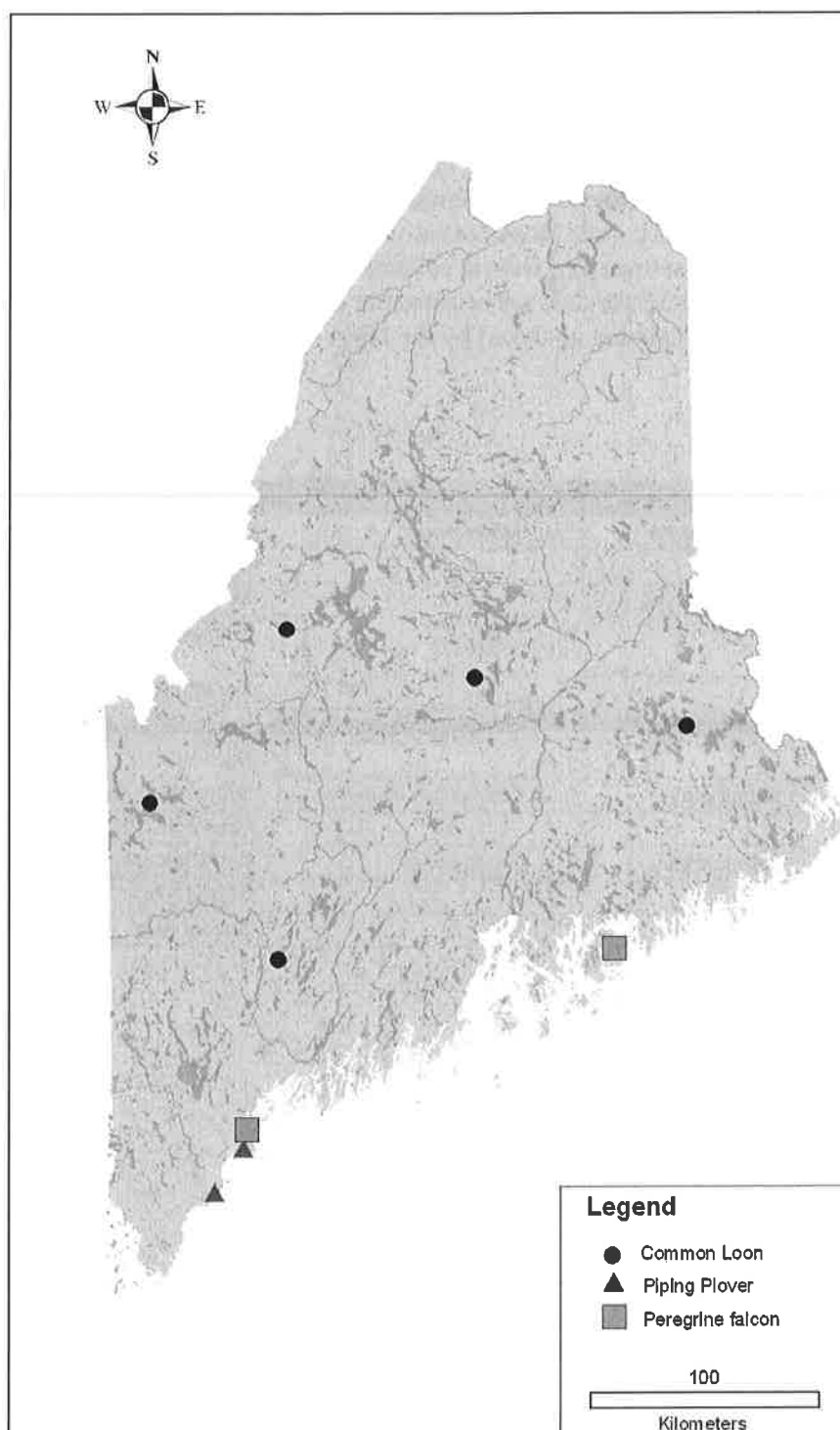
### 3. METHODS

#### 3.1 Field

We collected viable and nonviable eggs from each species (Table 1 & 2). The eggs were collected by collaborators and placed in polyethylene bags and sent with dry ice to the Wadsworth Center (New York State Department of Health) for analysis (see below for methods). BRI currently has state and federal collection permits. Note: the loon egg from Squaw Lake was excluded for the data set because the egg did not have complete contents. A substitute egg is being analyzed by the lab.

**Table 1. Samples collected.**

| State | Common Name      | Scientific Name           | Site                   |
|-------|------------------|---------------------------|------------------------|
| ME    | common loon      | <i>Gavia immer</i>        | Androscoggin Lake      |
|       |                  |                           | Brassua Lake           |
|       |                  |                           | Ebeemee Lake           |
|       |                  |                           | Mooselookmeguntic Lake |
|       |                  |                           | Sysladobsis Lake       |
|       | peregrine falcon | <i>Falco peregrinus</i>   | Casco Bay              |
| NY    | piping plover    | <i>Charadrius melodus</i> | Bar Harbor             |
|       |                  |                           | Scarborough Beach      |
|       | common loon      | <i>Gavia immer</i>        | Gooserocks Beach       |
|       |                  |                           | Dry Channel Pond       |
|       |                  |                           | Moss Lake              |
|       |                  |                           | Squaw Lake             |



**Figure 1. Sampling sites.**

### 3.2 Statistics

We performed statistics with JMP (SAS Institute Inc., 2001). Each egg composite was treated as a sample size of one. For analysis both 2007 and 2008 data was combined for common loons, peregrine falcons, and piping plovers. We sought spatial trends by mapping contaminant levels in piping plover, and common loon. The range of each contaminant was displayed in three categories determined by natural breaks within the contaminant range for each species. Trends were evaluated qualitatively.

### 3.3 Egg morphometric measurements

An hand-held caliper, capable of recording the 0.1 of a mm was used to determine the length and width. The egg length was measured from tip to tip of the egg. The width was measured from the widest point of the egg. A digital balance capable of weighing to the 0.1 of a gram was used to measure weight of the eggs with shell (whole egg) and without shell (content weight). Graduated measuring cylinders with Milli-Q water was used to determine the volume of eggs, determined as the volume of water displaced (recorded in ml). Developmental stage of the eggs were recorded as a ranking of the developmental stage of the embryo. An embryological development scale used for common loon and waterfowl eggs was used to assess the developmental stage and ranked as NA, 0,1,2,3,4, and 5 as below:

---

**NA (not assessable):** Developmental stage could not be determined. Contents were gray or yellowish-tan in color and typically had a foul smell. A darker color suggested some degree of development had occurred, whereas a yellow homogeneous liquid may be sifted through and if no dark spots or hardened areas were found we classified the egg as infertile (0).

**0:** No development was evident. Egg had a yellow/orange or yellow/tan yolk (intact or broken down into a liquid). A translucent jelly-like mass surrounded the yolk sac and showed no sign of embryonic development (e.g. mass not dark or hardened).

**1:** Embryo was viable (length was up to 1.5 cm). The jelly like mass (embryo) was dense and hardened. Small dark (red) eyespots may be visible at this stage.

**2:** Developing embryo (length was 1.5 – 2.0) has an apparent central nervous system. Cranial development and visible eyes are apparent. Feathers are absent.

**3:** The embryo shows advanced development (length was 2-3 cm). Bill was developed (e.g. egg tooth present but soft). Legs and wings were visible but not fully developed. Some feathers were present (first seen in tail).

**4:** The fully developed embryo was completely covered by feathers. Appendages were completely developed. Vent, preen gland was visible. A small portion of yolk sac remained attached to belly.

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### 3.4 Analysis of egg moisture and lipid contents

After the determination of morphometric parameters on each of the eggs, some samples collected from the same location and same species were pooled and homogenized using a homogenizer and composites were prepared. The composites were used for the analysis of trace organic contaminants and mercury. Homogenized egg samples (in most cases 10–11 g; for some samples only 5 g was used due to the availability) were extracted with dichloromethane and hexane (1:3; 400 mL) in a Soxhlet apparatus for 16 h after spiking the samples with surrogate standards (PCB-30 and PCB204). The extracts were concentrated to 10 mL and 1 mL of the aliquot was taken for the analysis of lipid content by gravimetry. An aliquot of the egg homogenate (approximately 2 g) was also taken and freeze-dried to measure the moisture content.

### 3.5 Analysis of PCBs, PBDEs and organochlorine pesticides

Details of the analytical methods have been described elsewhere (Kannan et al., 2005; 2007). An aliquot of the sample extract was spiked with  $^{13}\text{C}$ -labeled PCB congeners 3, 15, 31, 52, 118, 153, 180, 194, 206, 209, and  $^{13}\text{C}$ -labeled PBDE congeners 3, 15, 28, 47, 99, 100, 118, and 153 as internal standards. PCB congeners 30 (2,4,6-triCB) and 204 (2,2',3,4,4',5,6,6'-octaCB) were spiked as surrogate standards. The sample extracts were then purified by passage through a series of layers of silica gel (Davisil, 100–200 mesh, Aldrich, WI; 1 g of silica gel, 2 g of 40% acidic-silica gel, 2 g of 20% acidic-silica gel, and 1 g of silica gel at the top). The analytes were then eluted using 150 mL of 20% dichloromethane in hexane. The extracts were then concentrated using a rotary evaporator and treated with sulfuric acid (5 mL) and further concentrated to 1 mL for the analysis of PCBs and PBDEs. Another portion of the extract was passed through silica gel (2 g) by elution with 20% dichloromethane in hexane; it was then treated with sulfuric acid, for the analysis of organochlorine pesticides.

Extracts were injected into a gas chromatograph (Hewlett-Packard 6890) coupled with a mass-selective detector (Hewlett-Packard, series 5973) for the determination of PCBs and PBDEs. A capillary column coated with RTX-5MS (30 m x 0.25 mm i.d. x 0.25  $\mu\text{m}$  film thickness; Restek Corp, Bellefonte, PA) was used for the separation of individual isomers. The column oven temperature was programmed from 100°C (1 min) to 160°C (3 min) at a rate of 4°C/min, and then to 250°C at 3°C/min, with a final hold time of 5 min for PCBs. For PBDEs, the column temperature was programmed from 100 °C (1 min) to 160°C (3 min) at a rate of 10°C/min, and then to 260°C at 2°C/min, with a final hold time of 5 min. The MS was operated in an electron impact (70 eV), selected ion monitoring mode. An equivalent mixture of Kanechlor (KC300, 400, 500, and 600) with known PCB composition was used in the identification of PCB congeners. One hundred and fifty four isomers of PCBs with 35 coeluting pairs (IUPAC number in the order of GC-MS elution: 4+10, 9+7, 6, 5+8, 19, 18, 17, 15, 24+27, 16+32, 26, 25, 28+31, 20+33+53, 22, 36, 37, 54, 50, 53, 51, 45, 52+73, 46+69, 49+43, 47+48+75, 44, 59+42, 41+64, 40+57, 67, 63, 74+61, 70+76, 66+80, 60+56, 77, 104, 98+102, 93+95, 91, 92, 84, 90+101+89, 99, 86+97, 97+113, 87+117+125+116+111+115, 85+120, 110, 82, 124, 107,

118+106, 114+122, 105+127, 126, 155, 136, 151, 135+144, 149+139, 134, 133, 146+161, 153, 132+168, 141, 137, 130, 138+164+163, 158, 129, 128, 167, 156, 157, 169, 188, 179, 176, 178, 187+182, 183, 185, 174, 177, 171, 173, 172+192, 180, 193, 191, 170, 190, 189, 202, 201, 197, 200, 198, 199, 196+203, 195, 194, 205, 208, 207, 206, and 209), including mono-*ortho* PCB congeners (105, 118, 189) were analyzed.

Quantification of PCB congeners was based on external calibration standards containing known concentrations of di- through deca-CB congeners. Concentrations of individually resolved peaks of PCB isomers were summed to obtain total PCB concentrations. PBDE congeners were monitored at molecular ion clusters,  $[M]^+$  and  $[M+2]^+$  or  $[M+4]^+$ . Tri- through hexa-PBDE congeners analyzed in this study were 28, 30, 47, 66, 85, 99, 100, 138, 153, and 154 were targeted for analysis. Hepta- through deca-BDE congeners (183, 203, and 209) were analyzed using a Agilent Technologies 6890N gas chromatograph-electron capture detector (GC-ECD). PBDE congeners were quantified using an external calibration standard. Organochlorine pesticides were analyzed using a Agilent Technologies 6890N gas chromatograph-electron capture detector (GC-ECD; for HCH isomers) and a gas chromatograph (Hewlett-Packard 6890) coupled with a mass-selective detector (Hewlett-Packard, series 5973) for DDTs, chlordanes and HCB. A capillary column coated with DB-5 (30 m x 0.25 mm i.d. x 0.25  $\mu$ m film thickness) was used for the separation of pesticides. Concentrations were calculated from the peak area of the sample to that of the corresponding external standard. DDTs refers to the sum of *p,p'*-DDE, *p,p'*-DDT and *p,p'*-DDD; chlordanes to the sum of *cis*-chlordanes, *cis*-nonachlor, *trans*-nonachlor, and oxychlordanes; HCHs to the sum of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -isomers. PCB and PBDE congeners are represented by their IUPAC numbers.

### 3.6 PCB and PBDE quality assurance and quality control

The extraction, clean-up, and fractionation steps were evaluated by measurement of the absolute recoveries of the compounds spiked and passed through the entire analytical procedure. Mean ( $\pm$  standard deviation) recoveries of  $^{13}\text{C}$ -labeled PCB congeners #30, 118, 153, and 194 spiked into the samples were  $80 \pm 14\%$ ,  $82 \pm 17\%$ ,  $89 \pm 12\%$ , and  $91 \pm 14\%$ , respectively. Recoveries of surrogate PCB congeners CB-30 and CB-204 spiked into the egg samples prior to extraction were  $72 \pm 10\%$ . Mean ( $\pm$  standard deviation) recoveries of  $^{13}\text{C}$ -labeled PBDE congeners 28 and 47 were  $92 \pm 14\%$  and  $91 \pm 14\%$ , respectively. Overall recoveries of PBDEs ranged from 82 to 103%. The reported concentrations of PCBs, PBDEs and pesticides were corrected for the recoveries of surrogate standards (CB-30 and CB-204). Recoveries of organochlorine pesticides through the analytical procedure ranged from 85 to 110%. Procedural blanks were analyzed for every set of 10 samples, as a check for interferences. Calculated concentrations were reported as below the limit of detection, if either the observed isotope ratio was not within  $\pm 20\%$  of the theoretical-ratio, or the peak area was not greater than the specified threshold (3 times the noise). Known concentrations of PCBs, PBDEs, and organochlorine pesticides were spiked into selected samples (matrix spikes) and passed through the entire analytical procedures to calculate the recoveries. Recoveries of all of the target compounds spiked into egg matrixes were between 84 and 106% with a standard deviation of  $<15\%$ . The quantitation limits of individual PBDE

congeners varied from 10 to 500 pg/g, wet wt. The quantitation limit for organochlorine pesticides varied from 50 to 1000 pg/g, wet wt.

### 3.7 Analysis of perfluorinated compounds:

Potassium salts of PFOS (86.4%), PFOA (98%), PFOSA (95%), PFHxS (99.9%), and PFBS (99%) were provided by the 3M Company (St. Paul, MN). PFHpA, PFNA, PFDA, and PFUnDA were from Fluorochem Ltd ( $\geq 95\%$  purity, Derbyshire, UK).  $^{13}\text{C}_4$ -PFOS,  $^{13}\text{C}_4$ -PFOA (99% purity, Wellington Laboratories, Guelph, ON, Canada),  $^{13}\text{C}_4$ -PFNA and  $^{13}\text{C}_4$ -PFDA were used as internal standards and were spiked into egg samples prior to the addition of reagents for extraction.

PFCs in eggs were analyzed following the method described elsewhere (Tao et al., 2007). Egg homogenates (0.3-0.5 g) were taken in 15-mL polypropylene (PP) tubes and 5 ng of internal standards ( $^{13}\text{C}_4$ -PFOS,  $^{13}\text{C}_4$ -PFOA,  $^{13}\text{C}_2$ -PFDA, and  $^{13}\text{C}_2$ -PFNA), 2 mL of 0.25 M sodium carbonate buffer, and 1 mL of 0.5 M tetrabutylammonium hydrogensulfate solution (adjusted to pH 10) were mixed. Sample was then extracted with 5 mL of methyl-tert-butyl ether (MTBE) by shaking vigorously for 45 min. The MTBE layer was separated by centrifugation at 3500 rpm for 5 min and then transferred into another PP tube. The extraction was repeated twice with another 3 mL of MTBE. The MTBE extract was combined and evaporated to near-dryness under a gentle stream of nitrogen and then reconstituted with 1 mL of methanol. The sample was vortexed for 30 sec and filtered through a 0.2- $\mu\text{m}$  nylon filter into an autosampler vial. Matrix-matched calibration standards (seven points ranging from 0.5 ng/mL to 75 ng/mL) were prepared by spiking different amounts of calibration standards into a sample that contained no quantifiable amount of the target analytes; these standards were passed through the entire analytical procedure along with the samples.

Analytes were detected and quantified using an Agilent 1100 series high-performance liquid chromatography (HPLC) coupled with an Applied Biosystems API 2000 electrospray triple-quadrupole mass spectrometer (ESI-MS/MS). Ten microliters of the extract were injected onto a 50 x 2 mm (5  $\mu\text{m}$ ) Keystone Betasil<sup>®</sup> C18 column. The mobile phase was 2 mM ammonium acetate/methanol starting at 10% methanol, at a flow rate of 300  $\mu\text{L}/\text{min}$ . The gradient increased to 100% methanol at 10 min and was held for 2 min, and then reversed back to 10% methanol. The MS/MS was operated in electrospray negative ion mode. Target compounds were determined by multiple reaction monitoring (MRM). The MRM transitions were 299>80 for PFBS, 399>80 for PFHS, 499>99 for PFOS, 503>99 for  $^{13}\text{C}_4$ -PFOS, 599>99 for PFDS, 498>78 for PFOSA, 363>169 for PFHpA, 369>169 for PFOA, 372>172 for  $^{13}\text{C}_4$ -PFOA, 463>219 for PFNA, 513>219 for PFDA, 563>169 for PFUnDA, and 613>169 for PFDoDA. Samples were injected twice, to monitor sulfonates and carboxylates separately, and PFBS was monitored in both of the injections. A mid-point calibration standard was injected after every 10 samples to check for the instrumental response and drift. Calibration standards were injected daily before and after the analysis.

The egg samples were quantified with the quadratic regression fit analysis weighted by  $1/x$  of a matrix-extracted calibration curve. The limit of quantitation (LOQ) was determined as the lowest acceptable standard in the calibration curve that is defined as a standard within  $\pm 30\%$  of the theoretical value, and that has a peak area twice as great as the analyte peak area in blanks. LOQs for PFCs were 0.28 to 0.6 ng/g, wet wt, except for PFDS and PFBS, for which the LOQs were 0.94 and 1.12 ng/g, wet wt, respectively.

### 3.8 PFC quality assurance and quality control

Matrix spikes (6 egg composites) were performed for egg samples. Known amounts of mixed PFC standards (20 ng each) were spiked into sample matrices before extraction and were passed through the entire analytical procedure. Recoveries of PFCs spiked into egg homogenates and passed through the entire analytical procedure are shown in Table 3. The recoveries of all the PFCs were acceptable except for PFBS, which had a low recovery; however, PFBS does not bioaccumulate in tissues and also had not been detected in biological samples. Four  $^{13}\text{C}$ -labeled internal standards were spiked into all samples before the extraction, and the recoveries of internal standards are also shown in Table 3. Reported concentrations of PFCs in egg samples were not corrected for the recoveries of internal standards. Blanks were analyzed by passing Milli-Q water and reagents through the whole analytical procedure. Blanks contained trace levels of PFOA ( $<100$  pg). Reported concentrations for PFOA in egg samples were subtracted from the mean blank values. A midpoint calibration standard was injected after every 10 samples to check for instrumental stability, response and drift. Calibration standards were injected daily before and after the analysis.

### 3.9 Mercury analysis

Egg composites were freeze-dried and homogenized; an aliquot ( $\sim 0.1$  g) of the sample was weighed in a vial lined with Teflon®. Samples were digested overnight in concentrated nitric acid (2 mL). Samples were then further digested in a microwave oven for 7 min at 200 W; this step was repeated three times. Concentrations of Hg were determined by a cold vapor atomic absorption spectrometer (Model HG-3000; Sanso, Tsukuba, Japan). The limit of quantification was 50 ng/g, dry wt. Accuracy of the analysis was examined by analyzing Certified Reference Materials: dogfish muscle (DORM2; National Research Council, Ottawa, ON, Canada) and bovine liver (SRM1577b; National Institute of Standards and Technology, Gaithersburg, MD, USA) along with the samples. The overall analytical scheme used for the analysis of egg samples is shown in Figure 3.

## 4. RESULTS AND DISCUSSION

### 4.1 Relationship between compounds (Figure 2)

We found that with the additional samples added that PCBs, PBDEs, chlordane, and DDE continue to all significantly increase simultaneously ( $p < 0.0001$ ). When we analyze the common loon subset we also found a positive significant relationship, but not as strong. This finding indicates that birds with high PCB levels also tend to have high PBDE, chlordane, and DDE levels. This is consistent in studies conducted with OCs, which show that the pesticides are positively correlated in animal tissue (Blus 2003). This is of particular interest because in mice PCBs and PBDEs are demonstrated to interact, and together, at low doses can enhance developmental neurobehavioral defects (Eriksson et al. 2006). Additionally, researchers have also found that organochlorine pesticides (both DDE and chlordane are OCs) interact (Blus 2003).

The simultaneous increase in these compounds may be caused by a number of factors, including the similar chemical structure of PCBs and PBDEs, and their similar pattern of bioaccumulation. PCBs, PBDEs, and DDE are all composed of two benzene rings, but in PCBs the benzene rings are connected with a carbon bond, while in PBDEs there is an oxygen atom. PBDEs have attached bromine atoms, while PCBs have attached chlorines. This similar structure may mean that they move through the environment in a similar pattern.

PCBs and OCs have been extensively studied (Hoffman et al. 1996, Wiemeyer 1996, Blus 2003), but only recently have PBDEs been studied in wildlife. The positive relationship between these compounds suggests that species and geographic areas that have been documented to have high PCB levels may also have elevated PBDEs.

### 4.2 Hg (Figure 3, 4)

#### 4.2.1 *Comparison to known effects thresholds*

Two out of the 11 loon samples are above the known effects threshold of  $1.3 \mu\text{g/g}$  (ww, ppm) (Evers et al. 2003, Evers et al. 2007a) as well as one peregrine falcon egg. The eggs from Azischohos and Flagstaff were collected at sites known to have high Hg levels (BRI unpublished data) and in an area documented as a mercury hotspot (Evers et al. 2007b). Salamanders Hg levels are higher than other sites in Acadia National Park (Banks et al. 2005). All the piping plover levels were well below effects levels.

#### 4.2.2 *Comparison with other studies*

Our results are consistent with other studies (levels from other studies are bold and in brackets). Common loon eggs consistently have the highest Hg levels in multi-species

studies (Evers et al. 2005). However, our mean 1.03  $\mu\text{g/g}$  (both years combined), is higher than the regional mean [**0.78  $\mu\text{g/g}$  (ww)**](Evers et al. 2005)]; this is the result of sampling at areas known to be high. We collected samples at these sites to determine if other contaminants would also be elevated.

The piping plover samples have Hg levels consistent with regional means: piping plover in our study (both years combined), 0.18  $\mu\text{g/g}$  (ww) [**0.17  $\mu\text{g/g}$  (fww)** (Mierzykowski et al. 2004); **0.24  $\mu\text{g/g}$  (ww)** (Goodale et al. 2009)].

The peregrine samples (0.39  $\mu\text{g/g}$ , ww) were consistent with mean Hg levels for Maine bald eagles, 0.39  $\mu\text{g/g}$  (fw). (DeSorbo and Evers 2007). These peregrines on Mount Desert Island had slightly higher Hg levels may be caused by the peregrines feeding on terns on Petit Manan Island (C. Todd, Maine Department of Inland Fisheries, pers. com.).

#### 4.2.3 *Spatial Variation*

Mercury accumulates in the environment in hot spots, influenced by deposition patterns, watershed chemistry, food web dynamics, reservoirs, and point sources (Evers et al. 2007). Our results are consistent with this pattern. As discussed above, the elevated Hg levels in loons generally fall within established hot spot (Evers et al. 2007).

### 4.3 PCB (Figure 5,6)

#### 4.3.1 *Comparison to known effects thresholds*

The effects of PCBs on wildlife have been well studied (Blus 2003). Studies on bird eggs have shown chickens are particularly sensitive to total PCB levels and can show effects at 1,000-5,000 ng/g (ww) (Hoffman et al. 1996). In the field, total PCB levels have shown effects ranging from 8,000 – 20,000 ng/g in terns and other species (Hoffman et al 1996). Our results indicate that common loon eggs (total PCB 142 – 2719 ng/g, ww) are likely below adverse affects threshold. At this time I have not done an extensive analysis of coplanar PCBs, but the results can be seen in Table 2.

However, piping plovers at two sites, Ferry Beach (Saco) and Popham Beach (Phippsburg), have PCB levels greater than 1,000 and may be more sensitive to contaminants. Although effects thresholds have not been determined for piping plovers, a study conducted on a close relative, snowy plovers, indicates that contaminants could be among a number of stressors leading to the decline of least terns and snowy plovers (Hothem and Powell 2000). The authors did conclude, however, that the levels they recorded were not sufficiently elevated to cause concern. The level they recorded for total PCBs (330 – 2,360 ng/g, ww) in snowy plover are similar to our results.

#### 4.3.2 Comparison with other studies

Our mean loon total PCB level (1,136 ng/g, ww) is similar to that in Massachusetts 1,680 ng/g (ww)(Savoy 2004), but lower than those previously detected in Maine 0.91 ng/g (ww) (Mierzykowski et al. 2004).

Our total PCB, 1,406-5,908 ng/g (ww) in peregrine falcons, are generally lower than those detected in eagles in Maine between 1994-1996 (levels from other studies are bold and in brackets) [**330-45,398 ng/g (fww)(Matz 1998)**], but similar to levels detected along the Penobscot River, Maine [**6,230 – 11,410 ng/g (fww)(Mierzykowski and Carr 2002)**].

We found the piping plover total PCB range is 160-1,876 ng/g (ww), with some samples higher than detected in previous studies on Laudholm Beach [**560 ng/g (fww)(Mierzykowski and Carr 2004)**].

#### 4.3.3 Spatial variation

There is not a general spatial trend for either common loon or piping plovers across the state, with some of the most elevated levels in relative close proximity to lower levels. These results suggest that individuals' exposure can be dramatically different because of potential point sources, specific watershed characteristics, or food web dynamics.

### 4.4 PBDEs (Figure 7, 8, 9)

#### 4.4.1 Comparison to known effects thresholds

Laboratory study in kestrels found negative physiological effects in chicks that had 1,500 ng/g total PBDE injected into their egg and were fed 100 ng/g per day (Ferne et al. 2005). Our egg total PBDE residues, ranging 5-407 ng/g (ww), are not as high as the kestrel dosing study. Consequently, we do not know if the levels we recorded are having a negative effect. Of note was that the peregrine falcon on MDI had the highest total PBDE level of this new data subset, but was lower than the highest eagle recorded in Goodale 2008.

#### 4.4.2 Comparison with other studies

Our highest peregrine falcon level, 407 ng/g (ww) is higher than Norwegian [**155 ng/g (ww) (Herzke et al. 2005)**], and Swedish peregrines (Sellstrom et al. 2004), but were consistent with southern New England (Chen et al. 2007).

#### 4.4.3 *Spatial variation*

PBDEs are distributed across Maine in a similar pattern as PCBs and PFOS. There is not a general spatial trend for either common loon or piping plovers across the state, with some of the most elevated levels in relative close proximity to lower levels. These results suggest that individuals' exposure can be dramatically different because of potential point sources, specific watershed characteristics, or food web dynamics.

#### 4.4.4 *Congener patterns*

We detected deca-BDE (209) in three loon samples (Forest Ingalls Pond, Ebeemee Lake, and Coleman Pond), all three peregrine samples, and in one plover sample (Scarborough Beach). The mean peregrine level was nearly 10 times higher than the mean loon level.

Although BDE 47, 99, 153 made up the majority of the samples, there is great variation in the pattern between species (loons were dominated by 47, peregrines by 153, and plovers by 99). This indicates that PBDEs may be entering environment, dispersing, and bioaccumulating in different patterns between food webs and habitats. Research has demonstrated that bacteria can cause deca to breakdown into the more toxic lower brominated congeners (He et al. 2006); consequently the levels of tetra- and octa-BDE that we recorded may have originated from deca.

### 4.5 PFC (Figure 10, 11, 12)

#### 4.5.1 *Comparison to known effects thresholds*

PFCs have only recently been identified as a persistent bioaccumulative contaminant of concern. Consequently, few studies have been conducted on effects in bird eggs. However, a study that injected perfluorooctane sulfonate (PFOS) in white leghorn chicken eggs—known to be particularly sensitive to contaminants—determined, based on reduced hatchability, that the lowest-observed adverse-effects level (LOAEL) was 0.1 ug/g or 100 ng/g (ww). The species we studied may be either more or less sensitive than the chickens.

Six of our eleven loon eggs, one peregrine egg, and one piping plover sample have PFOS values above the LOAEL of 100 ng/g (ww). One sample of note is the loon from Androscoggin Lake which had PFOS levels 1.75 times higher than the next highest level on Flagstaff.

#### 4.5.2 *Comparison with other studies*



Only one study looked specifically at PFOS in bird eggs and our results are comparable (levels from other studies are bold and in brackets) to double-crested cormorants in the Great Lake region [**157 ng/g (ww), yolk**] and ring-billed gull [**67 ng/g (ww, yolk)**] (Kannan et al. 2001). As noted above whole egg contaminant levels are lower than yolk levels. The 2008 results are consistent with 2007's, with the note that both peregrine eggs from '08 had substantially lower levels.

#### 4.5.3 *Spatial variation*

PFOS are distributed across Maine in a similar pattern as PCBs and PBDEs. There is not a general spatial trend for either common loon or piping plovers across the state, with some of the most elevated levels in relative close proximity to lower levels. These results suggest that individuals' exposure can be dramatically different because of potential point sources, specific watershed characteristics, or food web dynamics.

#### 4.5.4 *Congener patterns*

Initially we have focused on PFOS because research has documented that this congener bioaccumulates (Kannan et al. 2002). Our results indicate that for loons, plovers, and peregrines that 30-70% of total PFC are comprised of PFOS—peregrines had the highest overall percentage.

### 4.6 Organochlorine pesticides (Figure 13, 14)

#### 4.6.1 *Comparison to known effects thresholds*

Although the OCs tested are present in all species (except HCH), the samples are well below known effects thresholds. HCH are not detected in any samples. This is consistent with other studies that have not detected HCH, because it has a short half-life (Blus 2003).

Our 2007 results showed that the HCB range for all species is 0.75 – 20.33 ng/g (ww), which is significantly below the effects threshold of 35,000 ng/g (ww) (Wiemeyer 1996). All the 2008 samples fell within this range with one exception, the MDI peregrine falcon egg, which was 44.21 ng/g (ww) and the highest level recorded in our studies. This result is further evidence that the overall higher contaminant levels detected in this egg may be caused by the peregrines feeding on terns—in the 2007 seabirds, and terns in particular, had the highest HCB levels (Goodale 2008).

Our 2007 results showed that the chlordane residues range for all species is 1.81 – 259.51 ng/g (ww), which is significantly below the effects threshold of 2,000 ng/g (ww) (Blus 2003). Our 2008 results also fell within this range with again the exception of the MDI egg which had the highest level we have recorded to date (329 ng/g, ww).

Our 2007 results showed that the DDE residues range for all species is 9.91 – 2,072.44 ng/g (ww), which is significantly below the effects threshold of 3,000 -30,000 ng/g (ww) (Blus 2003); however, studies indicate that slight egg shell thinning is possible at lower levels. Depending on the species, no eggshell thinning is seen below 100 to 2000 ng/g (Blus 1996). Again with one exception, the 2008 data fits within the range. The MDI peregrine egg however, has the highest DDE levels we have recorded and are potentially at a level that could cause limited egg shell thinning.

#### 4.6.2 *Comparison with other studies*

The levels of OC measured in our study are generally in the range detected in other studies (levels from other studies are bold and in brackets). Our HCB and DDE levels in piping plover are nearly identical to residues detected in a 2003 Maine study (Mierzykowski and Carr 2004).

#### 4.6.3 *Spatial variation*

DDEs are distributed across Maine in a similar pattern as PCBs, PBDEs, and PFOS. There is not a general spatial trend for either common loon or piping plovers across the state, with some of the most elevated levels in relative close proximity to lower levels. These results suggest that individuals' exposure can be dramatically different because of potential point sources, specific watershed characteristics, or food web dynamics.

#### 4.7 Overall conclusions

In 2007 we conducted a broad-based screening effort of contaminants to determine current levels of legacy contaminants (PCBs, and OCs) and emerging contaminants of concern (PBDEs and PFCs). The results from this first year indicate clearly that contaminants are both pervasive and persistent. Out of the 23 species studied in the first year, we determined that three required additional study in 2008: common loon, peregrine falcon, and piping plover. We selected these species because **loons act as bioindicators of lacustrine habitat throughout Maine, and peregrines and plovers are potentially at risk of bioaccumulating contaminants at levels above adverse effects thresholds.**

**Our 2008 results confirm our 2007 findings that both established (Hg, PCBs, chlordane, HCB, DEE) and emerging (PBDEs, PFCs) toxic pollutants of concern are bioaccumulating in Maine birds and that that PFCs are pervasive in Maine birds.** Prior to our study, PFCs had not been tested in Maine birds.

These results confirm that Maine common loons continue to be at risk from Hg and PFOS. With both contaminants, individuals had levels above suggested adverse effects thresholds. Of particular note is the one sample from Androscoggin Lake that had a PFOS level more than three times above the adverse effects threshold established for chickens (loons may be more or less sensitive than chickens). Although there are no published studies on interaction or synergy between PFOS and Hg, Hg has been shown to be synergistic with PCBs. Loons with high levels of both contaminants may be at higher risk of reduced productivity.

The peregrine samples show that there are between-year differences. This year we ran a second egg from South Portland and the contaminants during both years were within the same order of magnitude. With the exception of PFOS, the '08 sample tended to be higher. The differences between years may be attributed to the eggs being laid in a different order (for Hg, first-laid eggs tend to have higher levels than second-laid eggs), or dietary differences. The egg collected from Mount Desert Island has particularly high contaminant levels. The high levels in the MDI egg maybe attributed to the birds feeding on terns from Petit Manan Island, which would likely be at a higher trophic level than the South Portland birds that feed primary on rock doves. The terns could be accumulating contaminants in the wintering ground.

The two additional plover samples suggest that areas around Casco Bay may be higher in some contaminant levels. The sample analyzed from Scarborough Beach had the second highest PBDE, and the second highest DDE level. The sample from 2007 at Popham Beach had high PFOS and PCB levels. These differences could also be caused by local contaminant sources and birds feeding at different trophic levels.

Overall these data do not show a consistent spatial pattern for either the loons or plovers. These results suggest that individuals' exposure can be dramatically different because of potential point sources, specific watershed characteristics, and/or food web dynamics.

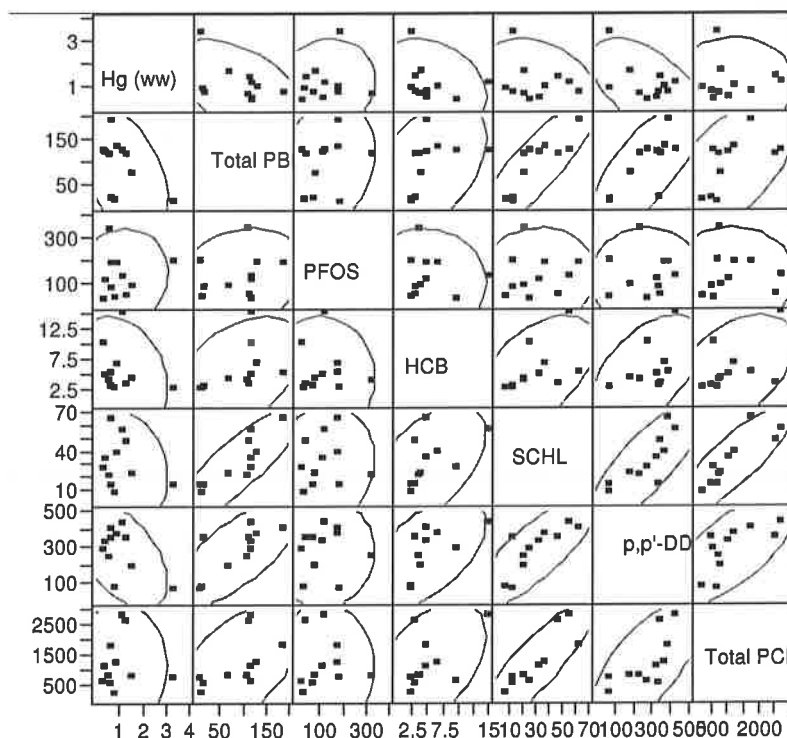
The 2008 results continue to show that many of the compounds we measured increase in concert with each other. One of strongest relationship we found is between PCBs and PBDEs, indicating that species and areas with high PCB levels may also have high PBDE levels. These relationships suggest that some species may have higher levels simultaneously of multiple compounds, which together may have greater negative impact on reproductive success, the neurological system, endocrine function, and overall physiology. Consequently, high trophic level predators may have a combined negative effect of these compounds despite having individual contaminants below known effects thresholds.

In summary, our results indicate that both historical and emerging chemicals of concern are accumulating in birds that forage in diverse ecosystems across the entire state of Maine.

## 5. ACKNOWLEDGEMENTS

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## 6. FIGURES AND TABLE



A

| Variable   | by Variable | Correlation | Count | Signif Prob |
|------------|-------------|-------------|-------|-------------|
| Total PBDE | Hg (ww)     | -0.0166     | 70    | 0.8916      |
| PFOS       | Hg (ww)     | 0.0249      | 70    | 0.838       |
| HCB        | PFOS        | -0.037      | 72    | 0.7577      |
| SCHL       | Hg (ww)     | 0.0533      | 70    | 0.661       |
| HCB        | Hg (ww)     | 0.0572      | 70    | 0.6381      |
| p,p'-DDE   | Hg (ww)     | 0.0933      | 70    | 0.4425      |
| Total PCBs | Hg (ww)     | 0.1316      | 70    | 0.2777      |
| p,p'-DDE   | PFOS        | 0.2011      | 72    | 0.0904      |
| HCB        | Total PBDE  | 0.2363      | 72    | 0.0457      |
| PFOS       | Total PBDE  | 0.2405      | 72    | 0.0418      |
| Total PCBs | HCB         | 0.3653      | 72    | 0.0016      |
| Total PCBs | PFOS        | 0.3716      | 72    | 0.0013      |
| SCHL       | PFOS        | 0.4054      | 72    | 0.0004      |
| SCHL       | Total PBDE  | 0.5344      | 72    | < 0.0001    |
| SCHL       | HCB         | 0.6493      | 72    | < 0.0001    |
| p,p'-DDE   | Total PBDE  | 0.5099      | 72    | < 0.0001    |
| p,p'-DDE   | HCB         | 0.5864      | 72    | < 0.0001    |
| p,p'-DDE   | SCHL        | 0.7689      | 72    | < 0.0001    |
| Total PCBs | Total PBDE  | 0.7543      | 72    | < 0.0001    |
| Total PCBs | SCHL        | 0.7406      | 72    | < 0.0001    |
| Total PCBs | p,p'-DDE    | 0.6097      | 72    | < 0.0001    |

B

| Variable   | by Variable | Correlation | Count | Signif Prob |
|------------|-------------|-------------|-------|-------------|
| Total PCBs | PFOS        | -0.0024     | 11    | 0.9943      |
| p,p'-DDE   | PFOS        | 0.0116      | 11    | 0.973       |
| Total PCBs | Hg (ww)     | 0.0148      | 11    | 0.9655      |
| SCHL       | PFOS        | 0.0848      | 11    | 0.8042      |
| HCB        | PFOS        | -0.0885     | 11    | 0.7959      |
| PFOS       | Hg (ww)     | 0.128       | 11    | 0.7077      |
| SCHL       | Hg (ww)     | -0.2272     | 11    | 0.5016      |
| PFOS       | Total PBDE  | 0.2405      | 11    | 0.4763      |
| HCB        | Hg (ww)     | -0.2444     | 11    | 0.469       |
| HCB        | Total PBDE  | 0.4429      | 11    | 0.1725      |
| Total PCBs | HCB         | 0.4934      | 11    | 0.123       |
| Total PBDE | Hg (ww)     | -0.4949     | 11    | 0.1217      |
| SCHL       | HCB         | 0.511       | 11    | 0.1082      |
| p,p'-DDE   | Hg (ww)     | -0.5378     | 11    | 0.0879      |
| p,p'-DDE   | HCB         | 0.5436      | 11    | 0.0839      |
| Total PCBs | Total PBDE  | 0.5674      | 11    | 0.0687      |
| Total PCBs | p,p'-DDE    | 0.6825      | 11    | 0.0207      |
| p,p'-DDE   | Total PBDE  | 0.7369      | 11    | 0.0097      |
| p,p'-DDE   | SCHL        | 0.7917      | 11    | 0.0037      |
| SCHL       | Total PBDE  | 0.8543      | 11    | 0.0008      |
| Total PCBs | SCHL        | 0.8659      | 11    | 0.0006      |

**Figure 2.** Correlation between compounds. In the graph, the stronger relationships have tight ovals while poor relationships have circles. The closer the correlation value is to 1 the stronger the relationship. Rows highlighted in gray are significantly related. A is the dataset as a whole, B is loon data only.

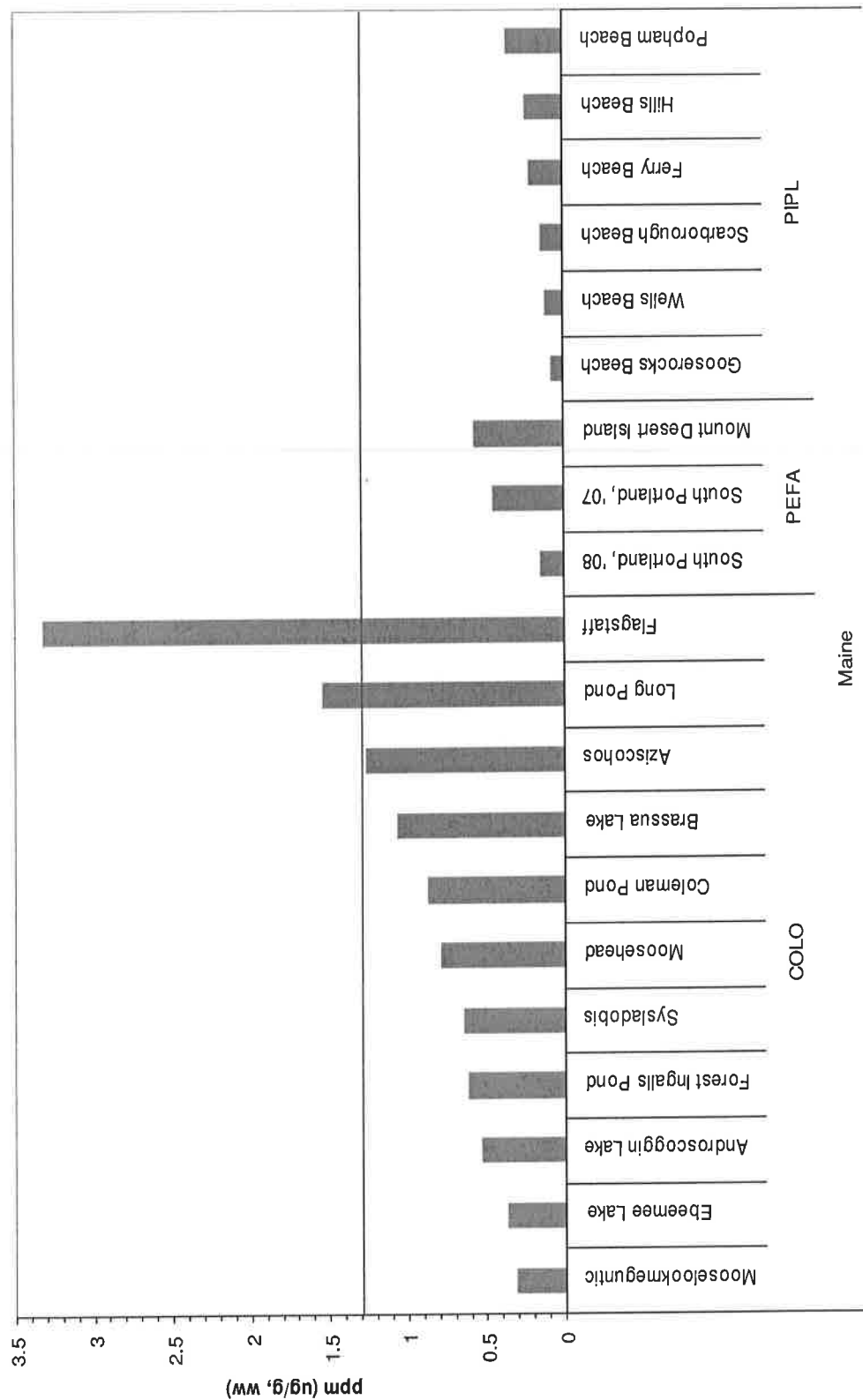


Figure 3. Hg Levels. Red line represents adverse effects level established by Evers et al. 2003.

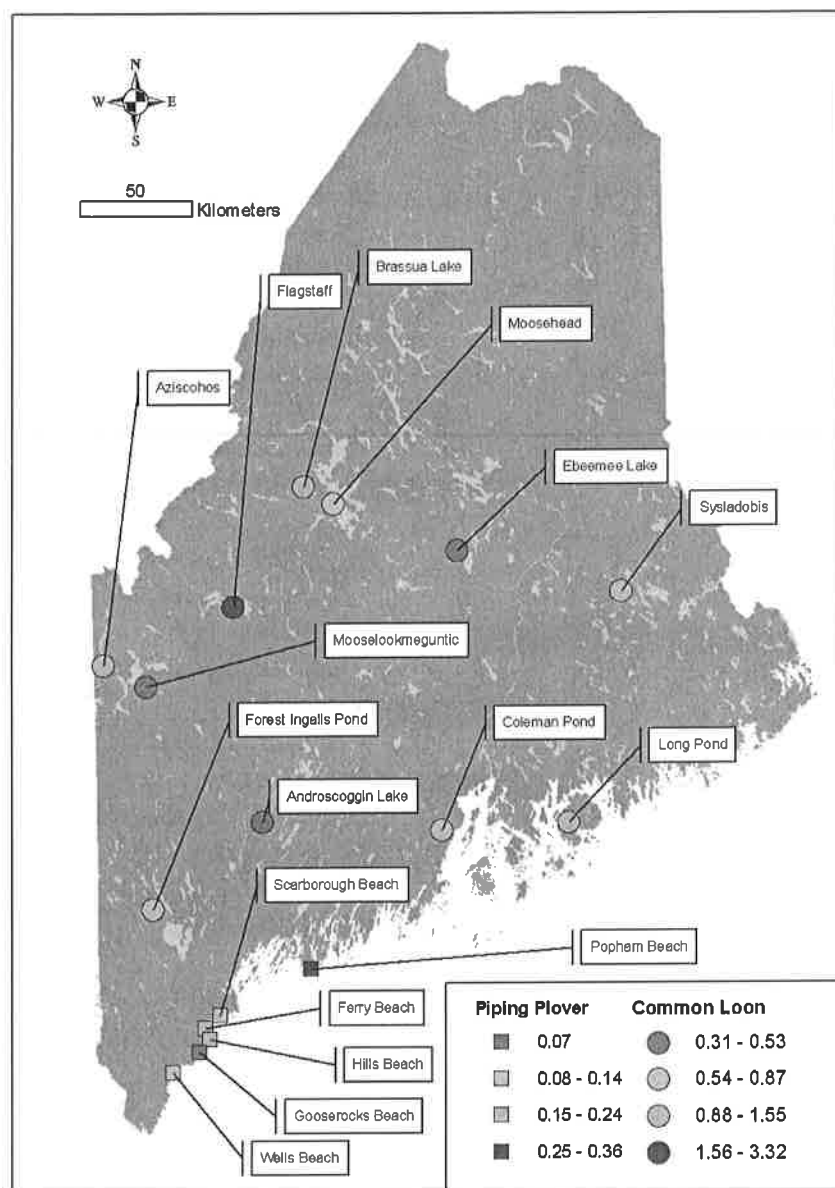


Figure 4. Map of Hg levels in common loons and piping plovers.

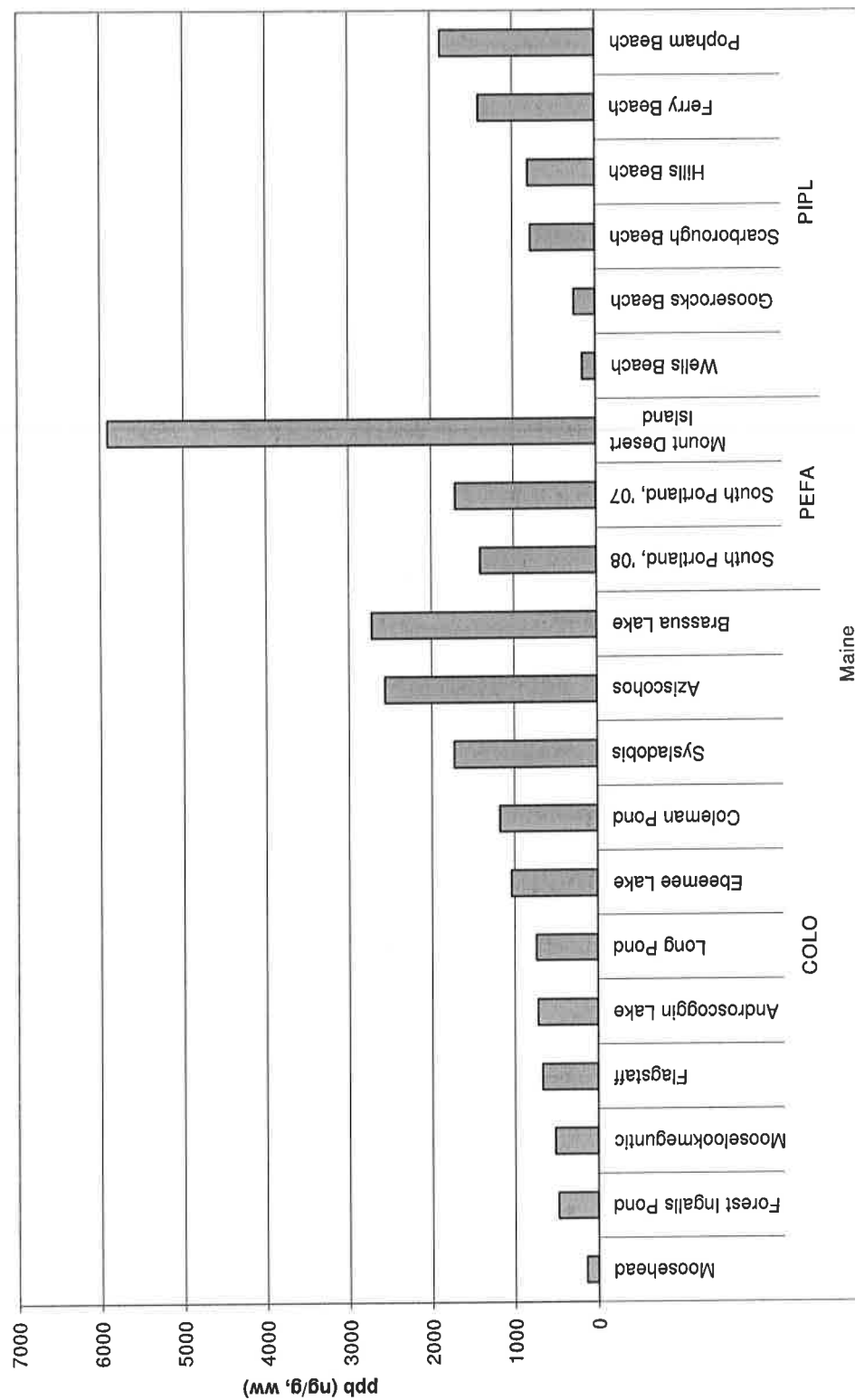


Figure 5. Total PCBs.



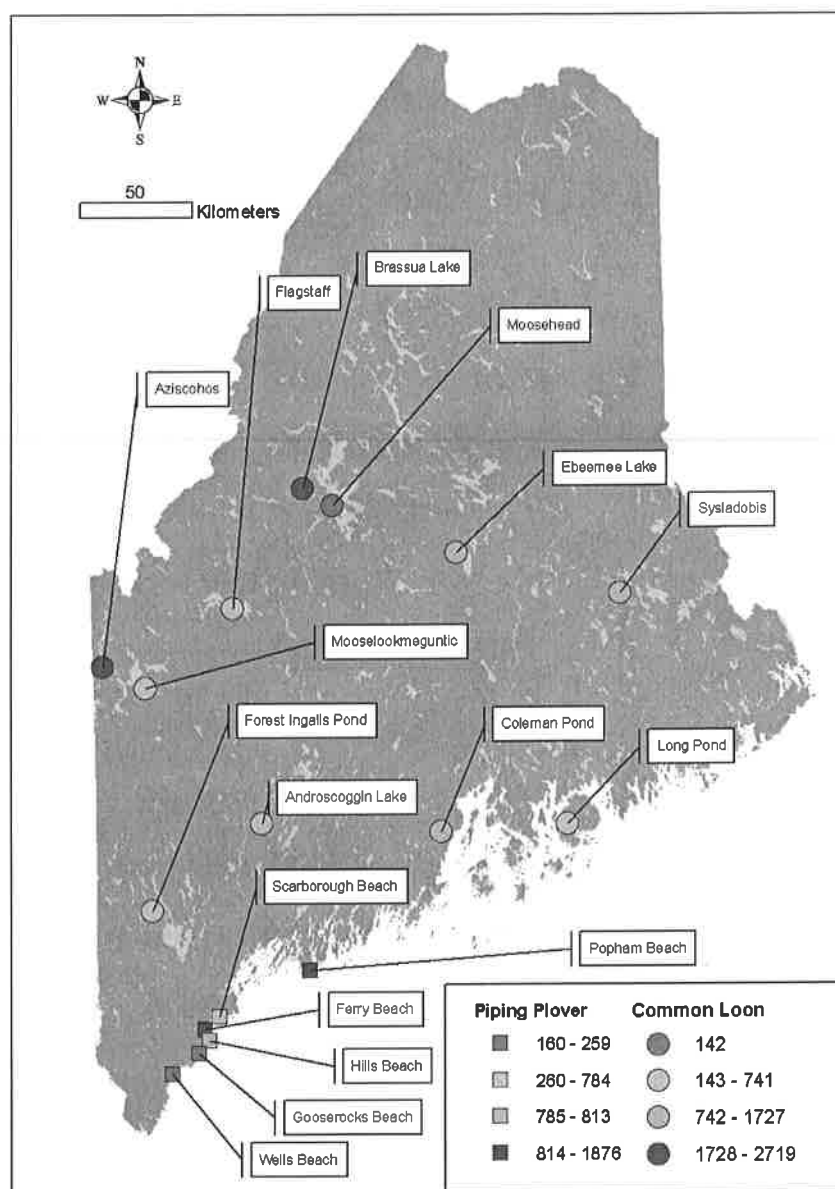


Figure 6. Map of total PCBs in common loons and piping plovers.

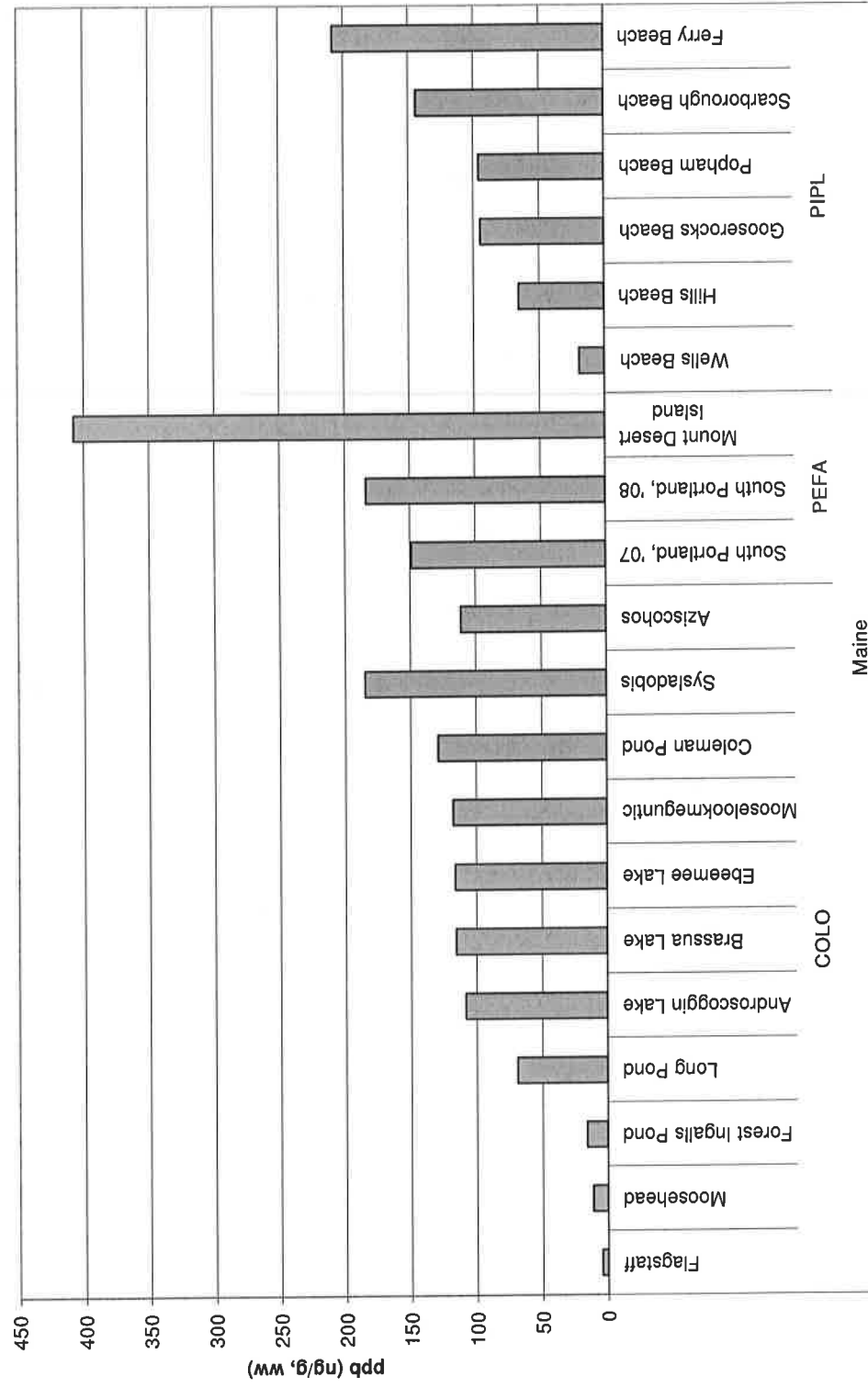


Figure 7. Total PBDEs.

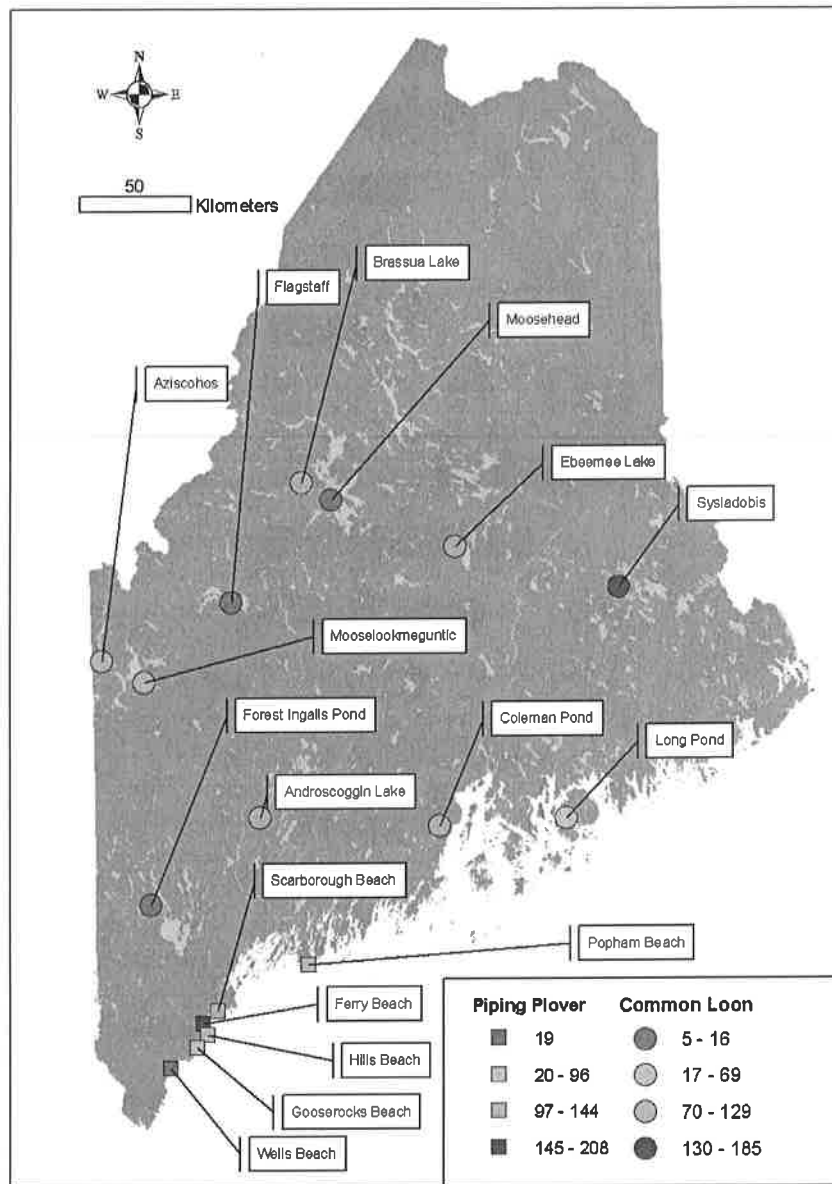


Figure 8. Map of PBDEs in common loons and piping plovers.

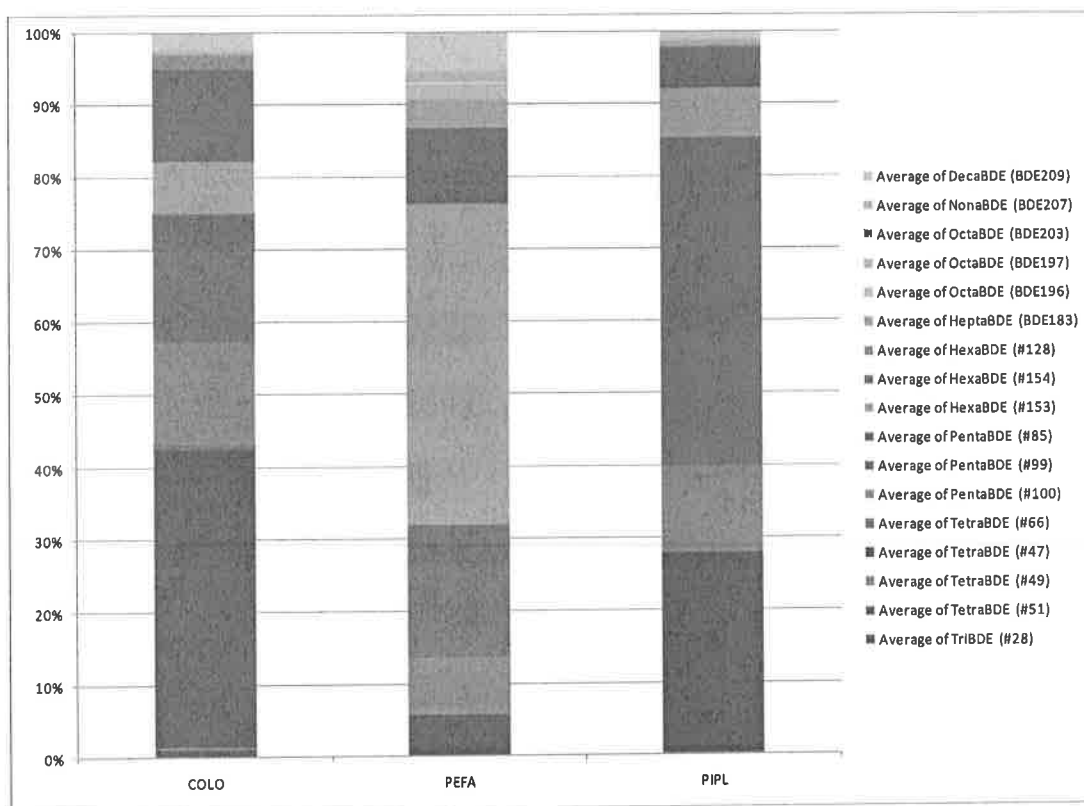


Figure 9. % of PBDE congeners.

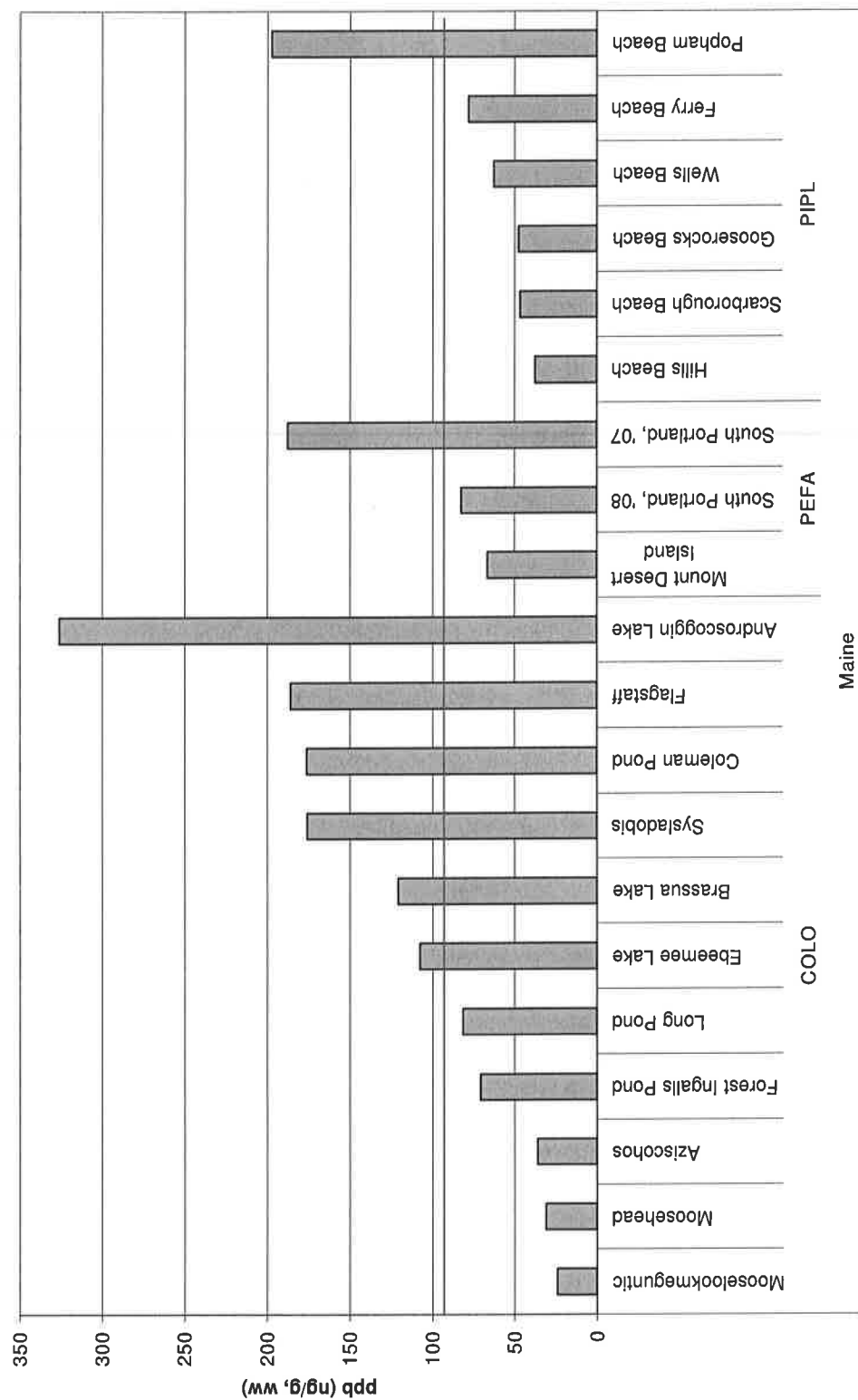


Figure 10. PFOS levels. Adverse effects threshold for chickens (Molina et al. 2006).

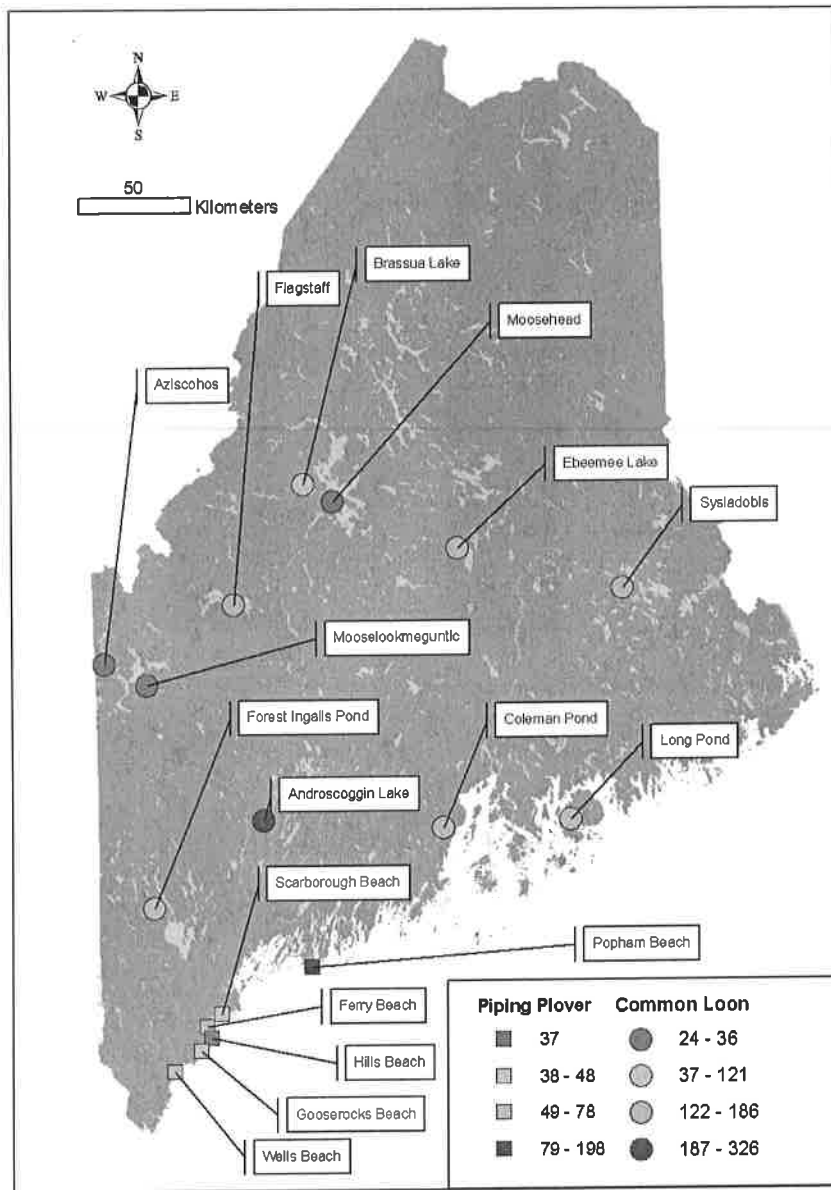


Figure 11. Map of PFOS in common loons and piping plovers.

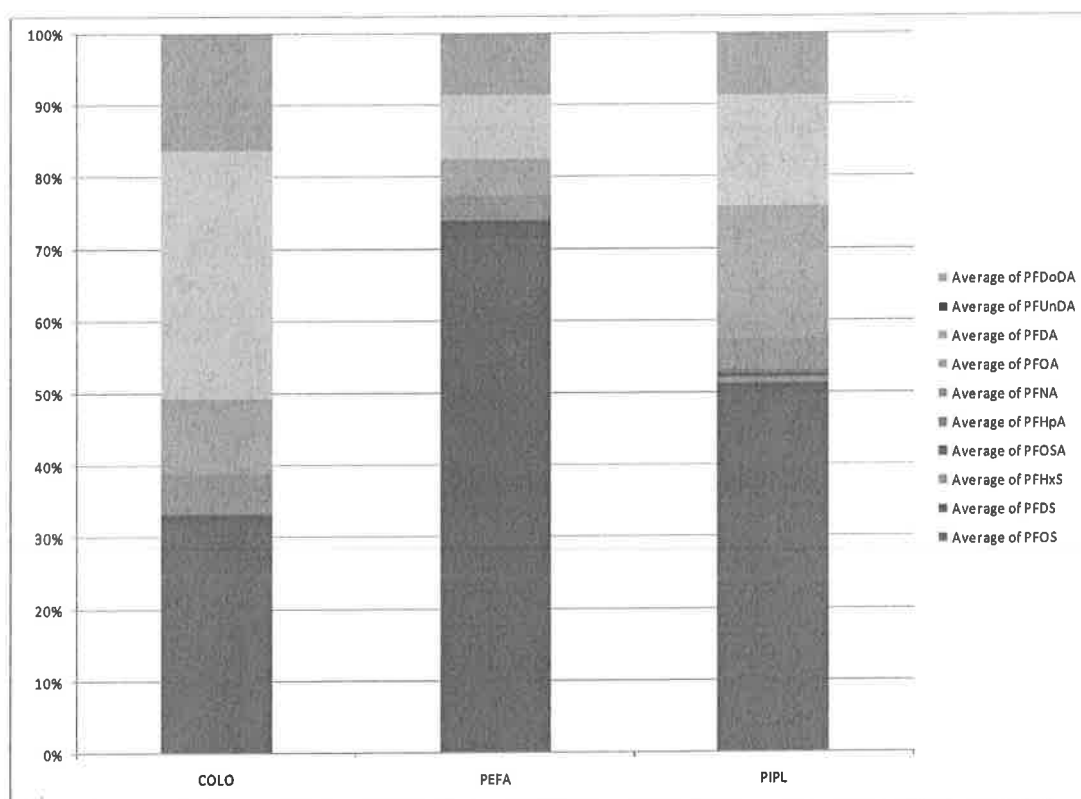


Figure 12. % of PFC congeners by species.

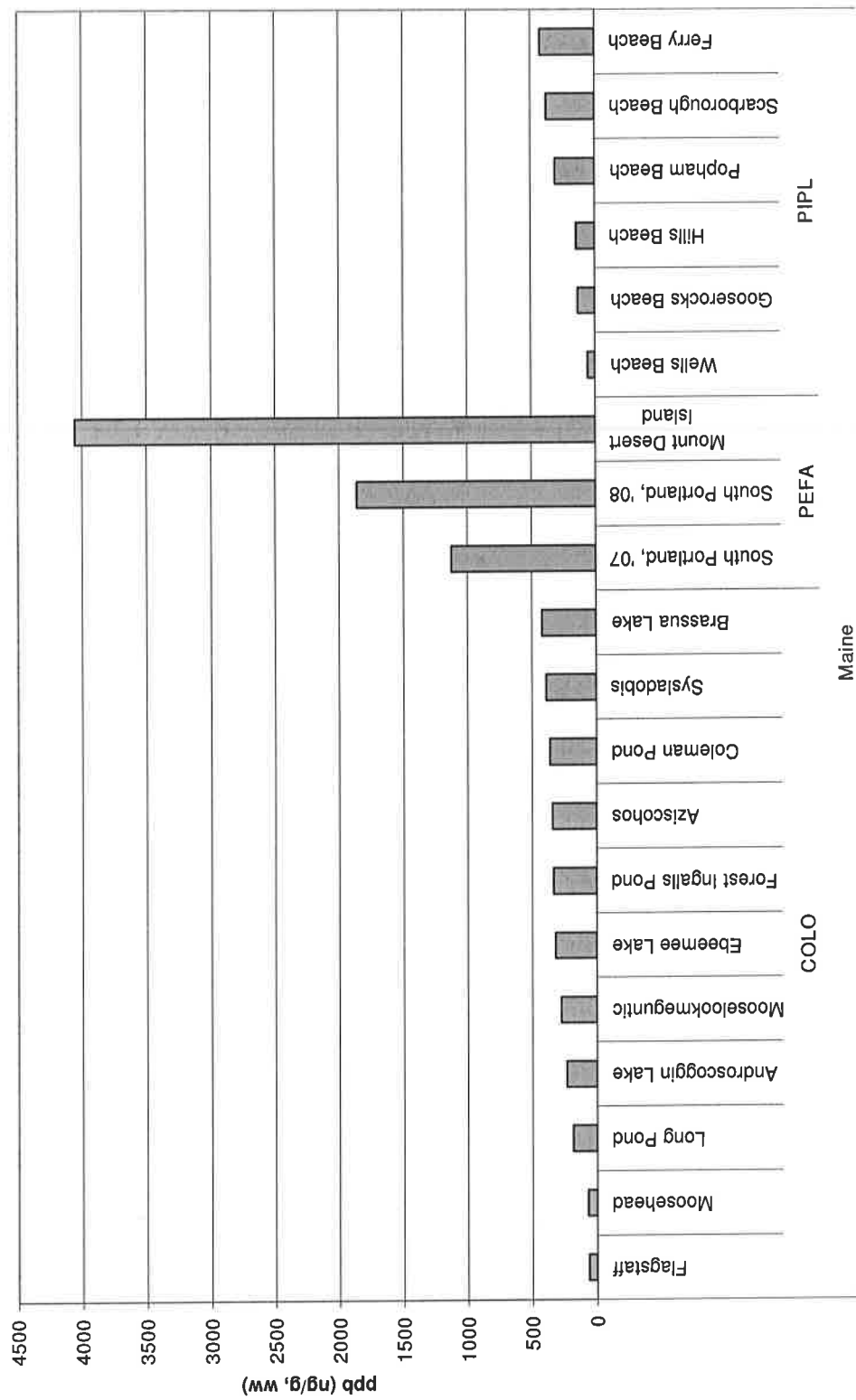


Figure 13. DDE levels.



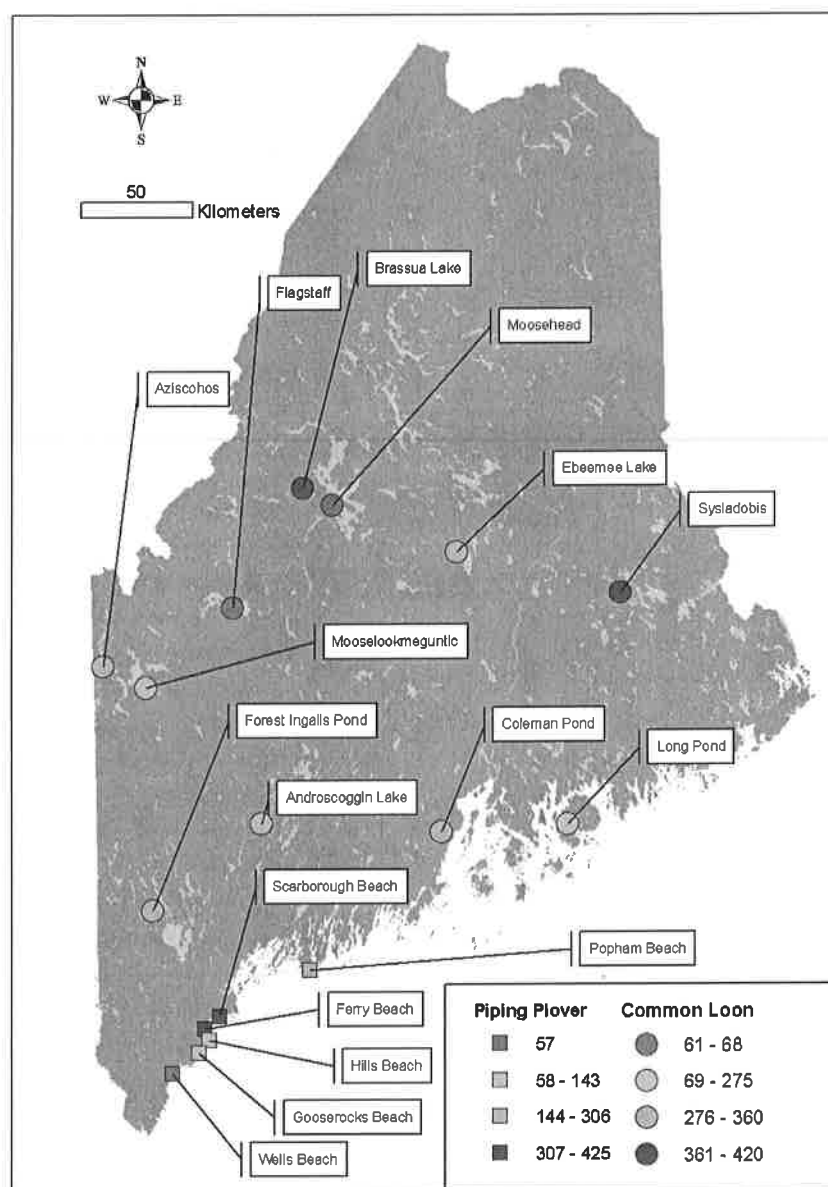


Figure 14. DDE in common loons and piping plovers.

Table 2. Table of samples.

| Composite # | Species          | State | Town/County     | Site                   | Date      | # composite eggs | composite | Development stage | Length (cm) | Width (cm) | Whole weight (g) | Content weight (g) | Volume (ml) | Moisture content % | Composite lipid content % |
|-------------|------------------|-------|-----------------|------------------------|-----------|------------------|-----------|-------------------|-------------|------------|------------------|--------------------|-------------|--------------------|---------------------------|
| Comp001     | common loon      | ME    | Wayne           | Androscoggin Lake      | 6/17/2007 | 1                | N         | 1                 | 9.43        | 5.85       | 161.3            | 134.8              | 170         | 72.7               | 9.62                      |
| Comp002     | common loon      | ME    | T6 ND BPP       | Sysladobsis Lake       | 6/30/2008 | 1                | N         | 1 (leak)          | 9.5         | 5.9        | 169.9            | 133.6              | N/A         | 74.6               | 11                        |
| Comp003     | common loon      | ME    | Long Pond TWP   | Brassua Lake           | 7/9/2008  | 1                | N         | 1                 | 9.63        | 5.56       | 161.5            | 136.1              | 165         | 71.8               | 10.8                      |
| Comp004     | common loon      | ME    | Ebeemee         | Ebeemee Lake           | 7/16/2008 | 2                | Y         | 2                 | 9.32        | 6          | 175              | 149.2              | 190         | 74.3               | 9.87                      |
| Comp004     | common loon      | ME    | Ebeemee         | Ebeemee Lake           | 7/16/2008 |                  | Y         | 1                 | 9.32        | 6          | 175              | 149.2              | 190         | 74.3               | 9.87                      |
| Comp005     | common loon      | ME    | Rangeley        | Mooselookmeguntic Lake | 8/4/2008  | 1                | N         | 1 (leak)          | 9.87        | 5.9        | 185.1            | 156.4              | 190         | 81.5               | 7.35                      |
| Comp006     | common loon      | NY    | Webb            | Moss Lake              | 6/28/2008 | 1                | N         | 1                 | 8.83        | 5.57       | 122.9            | 95                 | N/A         | 77.2               | 11.2                      |
| Comp007     | common loon      | NY    | Franklin County | Dry Channel Pond       | 7/7/2008  | 1                | N         | 4                 | 8.95        | 5.59       | 113.6            | 93.5               | 150         | 74.5               | 8.17                      |
| Comp009     | peregrine falcon | ME    | South Portland  | Casco Bay              | 5/6/2008  | 1                | N         | 3                 | 5.29        | 4.24       | 47.3             | N/A                | 45          | 83.8               | 3.93                      |
| Comp010     | peregrine falcon | ME    | Bar Harbor      | Precipice              | 5/1/2008  | 1                | N         | N/A               | 5.2         | 4.29       | 42.6             | 24.7               | 50          | 79.4               | 8.73                      |
| Comp011     | pipng plover     | ME    | Scarborough     | Scarborough beach      | 6/4/2008  | 2                | Y         | 1 (leak)          | 3.3         | 2.39       | 8.7              | 7.4                | N/A         | 65.4               | 20.0                      |
| Comp012     | pipng plover     | ME    | Kennebunkport   | Gooserocks Beach       | 5/30/2008 | 12               | Y         | 1                 | 3.13        | 2.39       | 9.2              | 8.1                | 9           | 68.9               | 15.8                      |
| Comp012     | pipng plover     | ME    | Kennebunkport   | Gooserocks Beach       | 5/30/2008 |                  | Y         | 3                 | 3.11        | 2.43       | 8.2              | 7.4                | 8           | 68.9               | 15.8                      |
| Comp012     | pipng plover     | ME    | Kennebunkport   | Gooserocks Beach       | 5/30/2008 |                  | Y         | 2                 | 3.3         | 2.54       | 9.8              | 8.6                | 9           | 68.9               | 15.8                      |

Table 3. Coplanar PCBs (ng/g, ww). Red values are the highest.

| Species | Site                              | Moisture (%) | Lipid (%) | PCB- 81 | PCB- 77 | PCB- 123 | PCB- 118 | PCB- 114 | PCB- 105 | PCB- 126 | PCB- 167 | PCB- 156 | PCB- 157 | PCB- 169 |
|---------|-----------------------------------|--------------|-----------|---------|---------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| COLO    | Androscoggin Lake, Inner Cove     | 72.7         | 9.62      | 0.007   | 0.006   | <0.001   | 0.510    | <0.002   | 0.148    | 0.044    | 0.114    | 0.140    | 0.044    | 0.018    |
| COLO    | Sysladobsis Lake, Burnt Island    | 74.6         | 10.95     | 0.070   | 0.021   | 0.036    | 1.551    | <0.002   | 0.403    | 0.295    | 0.254    | 0.221    | 0.111    | 0.093    |
| COLO    | Brassua Lake, Eagle/Center Island | 71.8         | 10.81     | 0.050   | 0.008   | 0.036    | 2.083    | 0.019    | 0.606    | 0.443    | 0.376    | 0.437    | 0.200    | 0.109    |
| COLO    | Ebeemee Lake                      | 74.3         | 9.87      | 0.039   | 0.004   | 0.009    | 0.360    | <0.002   | 0.090    | 0.188    | 0.052    | 0.053    | 0.015    | 0.062    |
|         | Mooselookmeguntic Lake, South     |              |           |         |         |          |          |          |          |          |          |          |          |          |
| COLO    | Toothaker                         | 81.5         | 7.35      | 0.015   | 0.006   | <0.001   | 0.582    | <0.002   | 0.142    | 0.082    | 0.108    | 0.093    | 0.044    | 0.047    |
|         | Casco Bay Bridge, South Portland, |              |           |         |         |          |          |          |          |          |          |          |          |          |
| PEFA    | ME                                | 83.8         | 3.93      | 0.052   | 0.459   | 0.041    | 1.924    | 0.014    | 0.456    | 0.185    | 0.354    | 0.498    | 0.125    | 0.082    |
| PEFA    | MDI, Precipice                    | 79.4         | 8.73      | 0.133   | 1.123   | 0.072    | 3.138    | 0.020    | 0.808    | 0.794    | 0.746    | 0.797    | 0.237    | 0.394    |
| PIPL    | Scarborough Beach                 | 65.4         | 20.00     | 0.036   | 0.387   | 0.031    | 1.418    | <0.002   | 0.326    | 0.155    | 0.208    | 0.257    | 0.078    | 0.026    |
| PIPL    | Gooserocks Beach                  | 68.9         | 15.76     | 0.014   | 0.151   | 0.010    | 0.158    | <0.002   | 0.032    | 0.064    | 0.025    | 0.032    | 0.011    | 0.015    |

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